ADHESIVE MICROVASCULAR ANASTOMOSES

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SIGNED DECLARATION

I declare that the contents of this thesis, submitted to the University of Edinburgh for the degree of Doctor of Medicine, were composed entirely by myself. The thesis is based entirely on my own observations and, except as indicated in the acknowledgements, the experiments were carried out, the data were collected and the results were analysed and interpreted by myself.

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ABSTRACT OF THESIS (Regulation 3.5.10)

Microvascular Surgery is a relatively new field of surgical reconstruction and is primarily practiced by Plastic, Orthopaedic and Hand Surgeons. Microvascular operations tend to be creative and unique, each being custom designed to solve a specific clinical problem. Three basic components are common to all microvascular operations: donor site preparation, recipient site preparation, and transplantation with microvascular anastomoses.

This thesis examines the use of adhesives in the fabrication of microvascular anastomoses.

Chapter 1 broadly examines the development, application and current practice of Microvascular Surgery. A historical review of vascular repair leads into a discussion of early and contemporary replantation and free tissue transfer operations.

Chapter 2 focuses on the technical aspects of microvascular anastomosis. A review of the many techniques used is followed by a description of the microvascular healing process.

Chapter 3 reviews the use of surgical adhesives. The characteristics of an ideal surgical adhesive are set out. Previous work with surgical adhesives is discussed, and the composition, mode of action, application and fate of fibrinogen adhesive is described in detail.

Chapter 4 describes a preliminary study in which a new adhesive microvascular anastomosis was developed. Using 6 rats and 12 rabbits an adhesive end-in-end technique was found to be easy to carry out and appeared to produce satisfactory vascular patency. A rabbit groin flap model was developed to test the new technique in a controlled manner.

Chapter 5 describes an experimental study to test the new technique in 50 rabbits using 100 consecutive flaps. Each animal acted as its own control: one flap had a sutured anastomosis and one flap an adhesive anastomosis. Despite numerous technical differences between adhesive and sutured anastomoses, flap survival and patency rates were not significantly different, although adhesive anastomoses were faster and less difficult. The healing of the two types of anastomoses was compared and contrasted using histological sections and scanning electron microscopy.

Chapter 6 examines the relevance of the adhesive technique to the contemporary Microvascular Surgeon, suggests other possible uses for fibrinogen adhesive in Microvascular Surgery, and speculates a little on future developments in this area of reconstructive surgery.

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PREFACE

Microvascular Surgery is a relatively new surgical subspecialty. It has revolutionised many areas of reconstruction, especially in the fields of Plastic Surgery, Orthopaedic Surgery and Surgery of the Hand. It has enabled dramatic improvements to be made in older surgical procedures, and has brought about the introduction of new operations that were previously not possible.

The ability to carry out successful microvascular repairs was the catalyst that made the new field of Microvascular Surgery possible.

The first microvascular anastomoses were made using suture technique. This method continues to be the most widely used and is generally considered to be the 'gold standard' to which any other method must be compared. It is, however, not without its own difficulties and problems, and it is for this reason that microvascular surgeons have continued to look for newer and better methods.

The purpose of this thesis was to review the development of Microvascular Surgery and to examine the variety of methods that have been employed for making microvascular anastomoses. Particular emphasis has been placed on the role of adhesives.

There are several reasons why this project was undertaken.

Over the years that I have been involved with Microvascular Surgery I have seen and heard of many different methods for making microvascular anastomoses. Some surgeons always use the same technique whereas others use varying techniques, depending on the circumstances. A few innovative surgeons are forever looking for newer and better methods. The majority, myself included, will fall somewhere in between, having a preferred technique but using other methods if the occasion demands. Hopefully we also maintain an open mind towards the use of newer methods as they become available.

A year or two ago, I was approached by a trade representative, from Immuno Canada, who asked if I had any use for their product,

a fibrinogen adhesive, marketed under the tradename Tisseel. Apart from the possibility of starting to use it for peripheral nerve repairs, I could not think of any particular application to the type of operations carried out in my practice of Surgery of the Hand and Orthopaedic Microvascular Surgery. The suggestion was made that I might consider using Tisseel for microvascular anastomoses. At the time I was unfamiliar with the literature concerning the use of adhesives in surgery, and my initial reaction was one of scepticism. In short, I thought that the idea of using a product, created from blood coagulation factors, anywhere near a microvascular anastomosis was absurd.

Nevertheless, I was intrigued by the idea. A review of the medical literature revealed that quite a lot had been written about the use of fibrinogen adhesives in surgery. Unfortunately the majority of the papers had been published in German, and the information they contained was not readily available to non German speaking surgeons like myself. I did have the opportunity to examine these references with a German speaking anaesthetist who picked out, and reviewed with me, the small number concerned with Microvascular Surgery.

The literature review did locate some articles, concerned with fibrinogen adhesive, in the English language literature. These led me on to papers concerned with the use of other adhesives in microvascular anastomoses. The reviews by Schlag and Redl[316,332], and Matras[244] were also particularly useful because they reviewed, in English, many of the papers written in the German literature. I cannot pretend to have read the German literature, but I have written and referenced the text in such a manner that the German literature can be easily located by an interested reader. In the reference section, I have included a few German papers, which I consider to be key articles, and the contents of which have been clearly reviewed by either the authors, or their colleagues, in English language papers.

After completing the literature review, I felt that, although there had been a moderate amount of interest in the use of adhesives for making microvascular anastomoses, there was plenty of room for further research because:

1. Non biological adhesives had been demonstrated to make technically good anastomoses, but these were unsatisfactory in the long term because of an intense inflammation reaction to the adhesive.
2. Use of the more promising fibrinogen adhesives was mainly documented in the German literature, which was essentially unavailable to English speaking surgeons.
3. The small number of studies published in English were not convincing as they were either too small to have statistical significance or they were carried out in a scientifically uncontrolled manner.

The literature also suggested that my initial scepticism might be unfounded, and that it might be possible to develop an adhesive microvascular technique using Tisseel. I also thought that if I was successful in developing a satisfactory technique for making an adhesive microvascular anastomosis, its description could be used as a medium for the publication of a review of the extensive German literature in an English language journal.

Thus, feeling that the full potential of adhesive microvascular anastomoses had not yet been explored, I set out to examine this whole area. The centre point of the task was to design and carry out an experimental study to investigate whether Tisseel could be successfully used to make reliable fibrinogen adhesive microvascular anastomoses. The experiment was designed to specifically answer the following questions:

1. Is there any statistically significant difference between the success of anastomoses made with fibrinogen adhesive anastomoses and those made with conventionally sutured anastomoses?
2. What morphological changes are seen at the anastomotic sites in anastomoses made with fibrinogen adhesive, and how do these compare with those at sutured anastomoses?
3. Are anastomoses made with fibrinogen adhesive faster, less expensive and less technically difficult than those made with conventional suture technique?

The thesis represents the full report of my investigations. It starts with a historical review of the development of Microvascular Surgery and goes on to discuss the different techniques and approaches that have been used for making microvascular anastomoses. Fibrinogen adhesive is next discussed in detail. A preliminary study, in which a technique for making fibrinogen adhesive microvascular anastomoses, is then described, and this is followed by a detailed account of a large, controlled, consecutive, and prospective experimental study designed to test the new technique. Finally an overall view of the role of fibrinogen adhesives in Microvascular Surgery is presented, and this is followed by a little speculative thought on the direction that Microvascular Surgery might take in the future.

CHAPTER 1

Development and Application of Microvascular Surgery

INTRODUCTION

In the past three decades, Microvascular Surgery has experienced an extraordinarily rapid growth and evolution. The field has progressed from a research curiosity to a clinical reality on a scale far beyond all expectations. Techniques are widely applicable and practitioners are drawn from many surgical specialties, but in particular from Plastic Surgery, Orthopaedic Surgery and Hand Surgery.

Microvascular operations are now routinely carried out at major hospitals around the world. Cases are done on both an emergency and elective basis. Results indicate that a high rate of microvascular patency is being achieved with marked and, often spectacular, improvement in the patients' clinical condition. The passage of time has shown that the procedures are reliable and can be reproduced by trained surgeons working in a variety of different locations.

Chapter 1 begins with a historical review of the development of Microvascular Surgery with emphasis on the introduction of the operating microscope and the development of microsutures, microinstruments and microsurgical technique. Next the application of Microvascular Surgery to replantation and free tissue transfer procedures is described. The chapter finishes with a discussion of current practice in a contemporary Microvascular Surgery Unit.

HISTORICAL REVIEW OF VASCULAR REPAIR

It has long been known that tissue viability is dependant upon an intact circulation of blood, with arteries transporting blood to the tissue and veins draining blood from the tissues.

Prior to the twentieth century, haemorrhage from a major blood vessel in an arm or leg was controlled by cauterization, compression or ligation. These techniques reduced the loss of lives but often resulted in the loss of limbs due to circulatory impairment. In those days attempts at limb salvage by surgical repair of blood vessels were associated with a high rate of wound infection - a frequently fatal complication.

Towards the end of the nineteenth century, largely due to the work of Koch and Lister, aseptic surgical technique became widely accepted. It then became possible to develop methods for the re-establishment of flow in severed blood vessels.

Some of the very early history of blood vessel surgery has been reviewed by Wintermantel[411]. Stromayr, in 1559, and Scultetus, in 1666, described surgical instruments and operative procedures for blood vessel surgery. In 1889, Jassinowsky, in his doctoral thesis, summarized the essential points of suturing arteries in animals. Abbe, in 1894, used glass tubes for vascular grafts in dog femoral arteries and cat aortae. In 1897, Nitze described an ivory prosthesis for vascular anastomoses. Also in 1897, Murphy reviewed the literature concerning vascular sutures and mentioned that Lambert, in 1762, pointed out the advantages of repairing, rather than ligating, damaged arteries. Murphy also described a new method for arterial repair that he called 'invagination', which was the first sleeve anastomosis. In 1899, Silberberg wrote a doctorate thesis on clinical and experimental research into vascular sutures.

At the turn of the century, Alexis Carrel[52] and other pioneering surgeons, such as Peyr, who suggested the use of magnesium tubes for vascular anastomoses, not only demonstrated the feasibility of vascular anastomosis with predictable patency rates, but also developed the techniques, some of which are still employed today.

Carrel was the first surgeon to apply the techniques of vascular repair to the transplantation of blood vessels, organs and limbs. Clinical successes in vascular surgery, however, were limited by crude suture material, inadequate needles, poorly designed instruments, traumatic tissue handling and continuing problems with infection.

Further progress was not made till after the introduction of sulphonamides in 1936 and penicillin in the 1940s. The large numbers of vascular injuries that occurred during the Second World War fostered considerable interest in the development of expertise in this area[93]. More sophisticated instruments were designed, and suture materials were developed to satisfy surgical demand.

The 1950s saw the operative repair of blood vessels become routine. During this period the results of seventy-seven consecutive acute vascular lesions occurring in Korean war wounds were published by Jahnke and Seeley[172]. The authors concluded that repair should be recommended in all cases of acute major arterial injury in order to decrease the incidence of amputation and also to maintain viability and function.

Repairs carried out more peripherally were still associated with a high incidence of intravascular thrombosis. At that time, it was not known why predictable anastomotic patency rates could not be achieved in vessels smaller than 2-3 millimetres in diameter. The solution to this enigma, however, was already developing in other surgical fields.

DEVELOPMENT OF MICROVASCULAR SURGERY

In the early days of Microvascular Surgery, several progress reviews were published[73,97,171,189,275,277,349].

Microscopes

The use of a microscope in surgery was pioneered by Nylén[271,272] in 1921. He used the magnifying power of a monocular microscope as an adjunct in operations on the middle ear, and particularly for otosclerosis. In 1922, Holmgren, Nylén's co-worker, developed a binocular operating microscope.

At first magnification was used to improve conventional procedures. Later it was discovered that the use of magnification allowed new operations to be developed for the small components within the ear.

In 1946, Perritt[275] started using the microscope for routine ophthalmic surgery. Ophthalmologists soon began to use microscopes to improve operations for the repair and reconstruction of the anterior chamber of the eye. The technique was widely adopted by eye surgeons in the early 1950s.

In 1953 Carl Zeiss commenced mass production of the modern operating microscope.

Microsurgical technique was applied to the repair of small blood vessels in experimental animals towards the end of the 1950s. The problems of small blood vessel repair were resolved with the adoption of this new approach, and in 1960 Jacobson and Suarez[168], using microsurgical technique, reported a 100% success rate in the anastomosis of arteries 1.4 millimetres in diameter.

From this point on the frontiers of microvascular surgery were pushed forward by reconstructive surgeons across the world. The foresight and tenacity of creative pioneers such as Acland (U.K.), Buncke (U.S.A), Chen (China), Cobbett (U.K.), O'Brien (Australia), and Yasargil (Switzerland), led to the successful realisation of operations hitherto only possible in dreams.

Some reconstructive surgeons focussed their attention on the application of microsurgical technique to the repair of peripheral nerves[348], whilst others continued to develop microvascular surgical techniques. Microneural Surgery has developed in parallel

with Microvascular Surgery, and is now well established and widely practiced[229].

The 1960s saw rapid development in instrumentation and technique, and surgeons began to report the results of repairing smaller and smaller blood vessels[1,110,164,361].

It soon became apparent that it was necessary to make adaptations to the binocular microscope to better suit the needs of the new microvascular surgeons. The need for the first assistant to actively participate in the process of microvascular anastomosis prompted the production of double binocular microscopes (sometimes called diploscopes). These enabled the surgeon and assistant, seated opposite one another, to see the same stereo image. Other improvements to the operating microscope have included:

1. Foot controlled, variable speed zoom focussing.
2. Foot controlled, variable speed zoom magnification.
3. X-Y axis motion with a foot controlled joystick.
4. Fibre-optic illumination.
5. A variety of balanced floor stands and ceiling mounts.
6. Tiny video cameras for closed circuit television monitoring.

These developments have provided modern microvascular surgeons with a choice of well suited and highly sophisticated microscopes for their work[201,202,293].

Microsutures

Traditionally sutures have been used by surgeons for the repair and reconstruction of damaged structures. In his 1976 Presidential Address, Snyder[352] said:

"Stitching is not new, for it was known the second week of creation. If you will open the Holy Bible to the book of Genesis, chapter 3, verse 7, you may read 'And the eyes of them both (Adam and his transplanted rib) were opened, and they knew that they were naked; and they sewed fig leaves together and made themselves aprons.' "

Historically as surgical technique became finer so smaller, and more delicate, suture materials were produced. It should not be surprising that the early microvascular surgeons wanted to use suture material for their microvascular anastomoses.

At first there were no satisfactory microsutures and experiments were carried out with various kinds of absorbable and non-absorbable suture materials[235,255,378]. Nylon (a generic name referring to a family of amide polymers) was found to be the best suture material as it was easy to use, of satisfactory tensile strength, and was found to cause very little tissue reaction. The production of ultrafine nylon suture material was relatively easy as the material could be spun to almost any diameter.

Needle construction was much more of a problem. Microneedles needed to be sharp tipped, smooth bodied, adequately shaped for ease of handling, and smoothly swaged to the suture material[307]. In the early days, small needles (often commercial ones individually reshaped and reground[49]) of different sizes, shapes and materials were tested but these were almost invariably too big and allowed for haemorrhage from large holes left in the vessel wall. A number of histological and scanning electron microscopic studies have documented the kind of vessel wall damage that is caused by microsutures[77,218,294,295,306].

Another approach was the development of metallised microsutures[46,276,280]. These were produced by depositing a fine film of metal onto the surface of a 12 micron nylon suture. They

were certainly small enough, but had the disadvantages of being too malleable, too blunt and too rough on the surface.

Commercially available microsutures were introduced by Ethicon with a 10-0 nylon on a BV2 needle in 1966. Further improvements quickly came from an industry that found it profitable to invest capital and personnel into a rapidly expanding subspecialty. The small S & T Chirurgische Nadeln firm (S & T after its founders W Springler and G Tritt) in West Germany proved to be particularly skilled in the production of microsutures. Working closely with Acland[3,7], they produced needles that were considerably sharper and smaller than those of their competitors. This was a great step forward because needle hole size, in the vessel wall, is an interplay of two factors: needle sharpness and needle diameter. The new S & T needles essentially solved the needle hole problem.

Modern microsutures are made straight, swaged onto the nylon suture material, and then curved by machine. The clear nylon is dyed blue, with methylene blue, or black, with logwood dye, to make it more visible. Needle points are extremely sharp, blades are tapered, bodies are smooth and fine, and the swages are secure. A variety of microsutures are available, although all are expensive because of the cost of labour for the difficult processes of sharpening and swaging. Most surgeons now use a 9-0 or 10-0 suture on a 100 or 70 micron needle for microvascular anastomoses, although an 11-0 suture on a 50 micron needle is available for very delicate work.

Microsurgical instruments

Use of the high magnification provided by the operating microscope, together with the production of ultrafine microsutures, necessitated the development of suitably sized instruments, specifically designed for the new microsurgical procedures. In many instances jeweller's and ophthalmic

instruments were adapted, but a number of completely new designs also emerged[8,277,85,282].

Standard surgical instruments are made with ring handles, and are manipulated by wrist rotation, chuck pinch and squeeze, using the thumb and ring finger. Microsurgical instruments tend to be more pencil shaped, and are manipulated by finger rotation and fine pinch between the thumb and index fingers.

Early on there was interest in externally powered devices driven pneumatically[328], hydraulically[46,361] and electrically[282,298]. In each case, however, it was found that these were complex to operate, slow, lacked precision and failed to improve the quality of anastomoses. Surgeons cast them aside in favour of the hand powered pinch controlled instruments[2].

Modern microvascular surgeons can choose their instrument sets from a wide array of commercial products, available largely due to the work of pioneering surgeons such as Acland, Buncke and Tamai.

Jeweller's forceps are used for tissue handling. These instruments come in various shapes and sizes and are classified according to the width of the bit (contact surface of the jaws), narrowness of the shank, and their overall configuration. Some have been modified for other applications: for instance an adaptation with smooth rounded jaws is used as a vessel dilator, and the No 2 jeweller's forcep is sometimes used as a needle holder[46].

Ophthalmic spring handled needle holders have proved to be very satisfactory for Microvascular Surgery. Fine tips are preferred, and a gentle curve serves to minimise obscuration of the surgical field. Ratchetless needle holders are used universally because their release is not associated with the violent movement of the ratcheted variety. Many surgeons use the needle holder for both passing the needle and tying the suture.

Microsurgical scissors have also been derived from ophthalmic surgery. Spring handled, round tipped, slightly curved

microscissors of the Westcott or Castroviejo type are commonly used for regular dissection. The smaller Vannas types are useful for fine dissection and suture cutting. Straight iris scissors are found in most sets and these, or a straight spring handled microscissors, can be invaluable in some difficult situations.

Large vascular clamps were developed for use in Peripheral Vascular Surgery. Smaller clamps were designed by neurosurgeons for the temporary occlusion of cerebral aneurysms. Clamps from both these sources have been used by microvascular surgeons, but a number of specialised microvascular clamps have also been produced[6,189]. Microsurgical clamps come in different widths and at different closing pressures. They are also available, mounted on a bar, as double approximating clamps[6,152,369]. Microvascular surgeons prefer to use fine clamps, which have a light closing pressure, in order to minimise endothelial damage[16].

Other instruments specifically needed for Microvascular Surgery include micro-bipolar coagulation, irrigators and small scalpels. Accessories that have been found to be useful are measuring devices, arteriotomy instruments[301], surgical background material, mobile arm rests[153], custom designed seats[283] and special operating tables[37].

APPLICATION OF MICROVASCULAR REPAIR

Microvascular Surgery has been applied both to emergency repair in the form of replantation and revascularisation, and to elective reconstruction in the form of free tissue transfer.

Replantation and revascularisation

The first application of small blood vessel repair was in the replantation and revascularisation of amputated parts. In 1960 Lapchinsky[203] obtained a 37% long term survival rate in limb

replantations in the dog. In 1963 Kleinert et al[187,188] described the successful revascularisation of partially amputated and non-viable digits. The following year Malt and McKhann[236] described two replantations of completely amputated upper extremities, and Horn[156] successfully replanted a hand. At that time it was felt that replantation was a reasonable option for certain patients in the right circumstances, although the results of experimental digital replantation were not consistently good[45]. In 1968 Komatsu and Tamai[195] reported the first successful microsurgical digital replantation after they reattached the thumb of a twenty-eight year old man who had been working with a steel cutting machine in Nara, Japan.

As clinical experience widened, the literature became replete with reports of replantation and revascularisation of digits and limbs. In the years that followed indications became more refined, technique and instrumentation improved, and more predictable microvascular patency was achieved. More recently, several studies have looked at the end functional results of replantation at different amputation levels[28,38,279,325,398]. These have been important papers as they have put the indications and long term goals of this new technology into perspective.

Surgeons in the People's Republic of China have shown great interest in Microvascular Surgery. Western surgeons have been fascinated by the Chinese experience partly because, until recently, political differences have prevented academic communication, and partly because of curiosity about the relationship of this modern technology with the practice of traditional Chinese medicine. The literature contains a number of papers describing Chinese microsurgical practice. These were mainly written with the specific intention of disseminating knowledge of activity in that country[58,80,296,318,415]. Chen et al[61], with multiple collaborators, have published a textbook, in English, describing Microvascular Surgery that has been carried out in China.

Chinese surgeons were experimenting with microvascular anastomoses[386] at the same time that reports were being published in the Western medical literature. They started carrying out replantations in the early 1960s. These first publications not only described replantation in the upper extremity[59,60,386,387], but also replantation in the lower extremity[159].

Free tissue transfer

The favourable results with replantation encouraged considerable research activity in laboratories around the world. Investigators quickly realised that many tissues could be totally isolated on vascular pedicles, transferred to distant sites and immediately revascularised to local arterial and venous channels using microvascular anastomotic technique. These procedures became known as FREE TISSUE TRANSFERS; the transferred tissue being removed from the DONOR SITE and revascularised in the RECIPIENT SITE.

Krizek et al[199] published the first experimental free tissue transfer in 1965. Soon after, Strauch and Murray[361] reported on their encouraging laboratory experiments. In 1966, Buncke et al[47] published the results of their work with toe-to-hand transplants in Rhesus monkeys.

At about the same time considerable interest was developing in the arterialised (axial pattern) flaps, that were later described by Smith[351] and McGregor[226], and comprehensively summarised by Daniel and Williams[82]. Microvascular surgeons thought that it should be possible to use these flaps for free tissue transfer, provided that the vessels in their vascular pedicles were large enough for microvascular anastomosis. In 1972, McLean and Buncke[230] carried out the first clinical free tissue transfer when they used a free omentum transfer to cover a scalp defect. The same year, Harii and Ohmori[144] transferred a hair-bearing cutaneous flap, based on the superficial temporal artery, to provide

hair for the opposite side of the scalp. In 1973, the first extremity reconstructions, using free cutaneous flaps, were reported from Melbourne[83,278].

Since the time of these original reports there have been rapid advances in this field. The development of myocutaneous flaps[222,223] considerably increased the number of free flap donor sites available and also led to improved knowledge concerning the value of muscle transplantation.

With such a large choice of donor tissues it soon became apparent that some transplants were better than others. Some free flap donor sites have stood the test of time and are still used a great deal (e.g. groin[83,225,278,350]; radial forearm[257,353,355]; lateral arm[180]; scapula[23,98,116,120,135,249,390,391]; parascapula[264]; dorsalis pedis[221,237,248,286,321]; latissimus dorsi[223,224,246,287]; gracilis[145,222,289]; rectus abdominus[39,42,51,303,376]), whilst others (e.g. axillary[25,35,112,372]; deltopectoral[20,21,226,143,144]; epigastric[15,83,165]; buttock[111]) have largely been superseded by better or more versatile flaps. There are some (e.g. thigh[354]; saphenous[12]) that, although technically satisfactory, have only found limited application and have never been used frequently.

To mention all the advances that have been made using the technique of free tissue transfer would fill a book in itself. In the reconstructive specialties, useful tissues that have been transferred include skin[82,142], muscle[242,222,368,238], fascia[41,166,210,241,377], tendon[285], bone[233,371,374,375,355], and nerve[373,228]. Composite free tissue transfers, such as toes[47,74], joints[161,284,414], epiphyseal plates[36,65,305], myocutaneous[222,223], and osteocutaneous flaps[233,355,374,375] have also proved to be useful.

As the new subspecialty developed, experience rapidly increased. The ability to freely transfer a donor tissue provided the ability to choose the best possible donor tissue to manage any particular clinical problem. As new techniques were explored, some of the patterns of reconstruction changed. For instance,

Head and Neck Surgery has been radically altered by the ability to reconstruct the mandible and floor of the mouth with osteocutaneous flaps such as the second metatarsal/dorsalis pedis flap, the osteocutaneous radial forearm flap and the osteocutaneous groin flap. Hand surgery has also been greatly improved by the ability to reliably carry out free flap or toe transfers as single stage procedures. In Orthopaedic Surgery, the management of Grade III open fractures of the tibia has been considerably enhanced by the use of free muscle and vascularised bone transfers.

The Chinese experience with microvascular free tissue transfers is now reasonably well known in the Western World. Less is known about the development of Microvascular Surgery behind the Iron Curtain. Some information is available in a recent publication[317] which presents the proceedings of an International Symposium on "Microsurgical Tissue Transplantation" which was held in Tübingen, Federal Republic of Germany, November 27-29, 1987.

THE NEW SUBSPECIALTY

The result of all this progress has been the emergence of a whole new subspecialty, which has become known as RECONSTRUCTIVE MICROVASCULAR SURGERY.

In any work, such as this, concerned with historical and technical aspects of Microvascular Surgery, some mention should be made of the process of dissemination of knowledge and skills from the pioneers to contemporary microvascular surgeons.

Microsurgical education

The early developments and technical successes were hard-won by a small number of surgeons working in laboratories across the world. Although much of the work was done personally by

these pioneers, valuable advice and technical assistance was also contributed by their colleagues, trainees and technical staff. The names of these individuals are recorded as co-authors on the relative scientific publications.

The first successful clinical microvascular cases were recognised as a tremendous advance in reconstructive surgery. Surgeons across the world rapidly became interested in learning the technique. Although much was to be learned by observing procedures in the operating room, this was clearly not the place to struggle with the difficult task of learning the delicate skills necessary to operate through the microscope and to successfully complete microvascular anastomoses.

Microsurgery teaching laboratories were established in the new centres. Trainee microsurgeons could either take short formal courses, or visit for more extended periods of time, advancing and perfecting their skills. Nowadays there are many laboratories[124] offering courses throughout the world. Excellent laboratory manuals are available, and these detail many of the technical skills that need to be mastered[11,50,335].

Well known centres have attracted many visitors. Some have wanted to stay for prolonged periods of time, and it was to satisfy this demand that Fellowship programs developed. These programs, usually lasting six months or one year, have allowed fully qualified reconstructive surgeons to acquire enough additional expertise to fully utilise the wide potential of Microvascular Surgery in their practices.

Microsurgical literature

Microvascular Surgery is now a well defined subspecialty of reconstructive surgery, although there may be considerable variation in interest between individual surgeons. Some devote the majority of their time to surgery of the hand whilst others are more concerned with reconstruction of the head and neck, the

extremities, children or have special interest in areas such as facial re-animation or tumour reconstruction.

A number of textbooks have been available since the early years[61,84,97,239,281,336,417]. Their content reflects the many facets of Microvascular Surgery and the wide knowledge of their authors.

The strength of Microvascular Surgery as a subspecialty is probably best illustrated by the many local, national and international societies which are thriving and providing a forum for the exchange of ideas. Abstracts and papers on microvascular subjects are published in a wide variety of scientific journals, but are generally to be found in those journals related to reconstructive surgery. In the English language literature the 'Journal of Reconstructive Microsurgery' and 'Microsurgery' are devoted exclusively to papers on topics related to the field of Microvascular Surgery.

Cost/benefit of Microvascular Surgery

It was interesting to note that as the new subspecialty emerged, there was considerable concern about its cost/benefit ratio. This issue remains relevant in today's climate of health care economics.

In 1979, McGrowther[227] won the Kay-Kilner Essay Competition with an entry entitled "The operating microscope: a necessity or a luxury?" He stated that the new field of microsurgery should be evaluated by answering certain questions:

1. What does the operating microscope enable us to do that we cannot do by alternate methods?
2. If there are alternate methods, do microsurgical techniques improve the results?
3. Do the results justify the investment of instrumentation, operating theatre time, man hours, and hospital inpatient time?

He concluded that, in his experience, microsurgery was clinically important, procedures utilised less hospital bed time than alternate methods, and that the technique should become an essential part of surgical training.

O'Brien[274] was of the same opinion. He commented that Zeiss microscopes, bought many years previously, were still in daily use in his unit, and, although improved in design, modern microscopes were not greatly changed from the early models. His experience was that hospital bed occupancy per patient for a one stage free tissue transfer in Melbourne, was less than for a multistaged pedicle flap reconstruction. In addition, he said that although operating time was greater for microsurgical operations, it was no more than the accumulated time used for multistaged procedures.

Serafin published figures[337] to support his opinion that the cost/benefit of Microvascular Surgery was good. In a comparison of several statistics relating to reconstruction of the head and neck, he demonstrated that total anaesthetic time was almost the same regardless of the type of flap used, but that microvascular cases needed less operations and considerably fewer days in hospital. Final success rates compared favourably with older forms of management.

Serafin's comparative statistics[334] for coverage of defects in the leg and foot conveyed the same message. Total expenditure was calculated for these cases, and the figures indicated that microvascular reconstruction was the least expensive method.

In Europe, Sykes and Bryson[366] studied a combined series of patients from England and Italy. Their conclusions were similar to those of Serafin.

CURRENT PRACTICE IN MICROVASCULAR SURGERY

Microvascular surgery is now widely practiced around the world. The specialty has tended to remain located in major surgical centres. There are many reasons for this, but, primarily, it is because the best results can be achieved using a team approach.

In a typical centre, the microsurgical team consists of three or four microvascular surgeons, a similar number of fellows (fully qualified surgeons), residents (at different grades), interns, clinical clerks and medical students. During operations, dissection of donor and recipient sites are carried out simultaneously by two teams. Later, revascularisation of the transferred tissue and donor site repair are also carried out simultaneously. This team approach minimises the operative time, and this results in a number of advantages: maintenance of the patient's body temperature, less fluid loss, shorter anaesthetic, less problems with surgeon fatigue, and the use of experienced daytime operating room staff for the whole case. Knowledgeable anaesthetists, experienced operating room and ward nurses, and specialised hand therapists, all contribute to a successful outcome.

The modern microvascular operating room is equipped with an extremely versatile operating diploscope, which is usually mounted on a counterbalanced floor stand, but may be on a fully tracking ceiling mount. It has tiltable binocular tubes, a tiny television camera (linked to a television monitor in the operating room), and foot controls operating the X-Y coupling, zoom focus, and zoom magnification.

The development of specific areas of interest amongst individual surgeons in the group allows for increasingly sophisticated procedures to be carried out. A contemporary group of microvascular surgeons has the ability to apply microvascular technique to the management of congenital anomalies, extremity reconstruction (bone or soft tissue for trauma or tumour) head and

neck reconstruction (bone or soft tissue for trauma or tumour), hand problems (emergency or elective), cosmetic problems, and areas of special interest such as functioning muscle transplantation for upper extremity trauma and facial paralysis, or the transfer of viscera such as bowel and testis.

CHAPTER 2

Microvascular Anastomosis: A Review of Surgical Technique and the Healing Process

INTRODUCTION

In Chapter 1 a broad descriptive picture was painted of the development, application and contemporary practice of Microvascular Surgery. The section on microsutures might have suggested to the reader that suturing is the method of choice for making microvascular anastomoses. There is no doubt that suturing is the most common method used at the present time, but for many reasons there has been, and still is[131], considerable interest in finding an alternative and/or better method. Some techniques have been tested and failed, others have been more successful, but comparatively few have survived the test of time to remain in common use.

Chapter 2 begins by classifying and reviewing techniques that have been used, both experimentally and clinically, for making microvascular anastomoses. Next the biological healing process of microvascular anastomoses is examined from the point of view of patency, histological changes and scanning electron microscopic appearance. The final part of the chapter briefly looks at the blood clotting mechanism and reviews many of the factors related to anastomotic failure.

TECHNIQUES USED FOR MICROVASCULAR ANASTOMOSIS

Historically, three factors proved to be essential for the successful repair of blood vessels 1.0 millimetre or less in diameter:

1. Magnification with the operating microscope.
2. Delicate instruments and tissue handling.
3. A satisfactory technique for microvascular coaptation.

Chapter 1 describes how these hurdles were crossed by either using modifications of known technology or developing new technology. It has always been felt that there must be a better way of achieving microvascular coaptation, and surgeons continue to strive for new and improved methods.

A large number of different techniques have been investigated for making microvascular anastomoses. Broadly speaking these can be thought of as falling into two main groups according to:

1. Method of fixation. This group includes sutures, laser techniques, electrocoaptation, mechanical devices and adhesives.
2. Type of anastomosis. This group includes end-to-end, end-to-side, end-to-side branch, end-in-end, cuffing techniques and size discrepancy.

Method of fixation

Sutures

The technique of suturing microvascular anastomoses has been widely used since Jacobson and Suarez[168] reported their successful results in 1960. Suture technique has made great progress[211] since that time and is still the most widely used method.

In a sutured microvascular anastomosis the surgeon should aim to achieve a leak-free anastomosis with as few sutures as possible. Adherence to these principles[150] minimises problems associated with medial necrosis and arterial occlusion. Sutures should pass through the full thickness of the vessel wall as this probably causes less media disruption than sutures which pass only part way through the wall[150]. The vessel ends should be closely approximated, and care should be taken that the tissue encompassed by the sutures is not strangulated[150]. It is standard practice to cut the ends of the vessels to be anastomosed squarely

across, but some have favoured oblique sectioning of the vessel ends[164].

Good technique is important[385] because technically poor suturing results in narrowing of the lumen at the anastomotic site, distortion of the vessel wall, and an increase in the likelihood of vascular thrombosis[16,253]. Sutures must be placed evenly and spaced correctly[320].

There are different methods for inserting the sutures. In order to avoid catching the back wall during suture placement, Cobbett[139] described an eccentric biangulation technique in which the initial two stay sutures were placed 120^{o} apart, whilst Daniel and Terzis[86] recommended using the triangulation technique where three stay sutures were placed 120^{o} apart. These techniques were not always easy to use because it was difficult to accurately place the stay sutures, particularly if there was a size discrepancy. Nowadays, most surgeons prefer a biangulation technique, using two stay sutures 180^{o} apart, although some surgeons use a successive interrupted ("ship's wheel" type) suturing technique[139]. Sutures should be tied with a conventional three throw surgeon's knot.

The distance between interrupted sutures should be separated by about 0.3 millimetres, or eight stitches for a 0.9 millimetre[150] or 1.0 millimetre vessel[63]. It is suggested that sutures are placed 60 to 80 microns[150] or 0.2 millimetres[63] from the edge of the divided vessel, although Harashina et al[136] could not demonstrate any difference in patency rates between anastomoses completed with small suture bites and anastomoses completed with large suture bites.

Studies have been carried out to compare different suturing techniques. Shumacker[341], in pre-microsurgical days, reviewed the literature on sutured vascular anastomoses, and described an experiment comparing the reliability of vascular repairs made with mattress and plain sutures, inserted using either interrupted or

continuous technique. He felt that mattress techniques would prove to be the most useful, but was concerned about the high rate of complications in small vessels. Microsurgical continuous suture technique[169,258] has been studied[32,131,134,140]. Patency rates were comparable to interrupted technique and the anastomoses were completed quicker, but the investigators felt that the problems associated with entrapment and breakage of the suture material on the microvascular clamps, necessitating repetition of the anastomosis, outweighed the advantages.

Others have been more optimistic about continuous suture technique[403]. Lee et al[212] proposed an open-loop technique for inserting a continuous suture and said that they did not run into technical problems.

Some researchers have investigated the use of different types of suture material. Phelan et al[304] compared sutures made out of silk, nylon and dacron. They demonstrated that dacron and nylon were superior to silk, but experienced difficulty tying the knots. Anastomoses with 9-0 dexon microsutures were studied by Peminello et al[302], and absorbable and non absorbable 10-0 sutures were compared by Mii et al[255], Mallon et al[235] and Thiede et al[378]. Each of these groups concluded that the absorbable sutures were very satisfactory, yet the use of absorbable sutures has not been widely adopted. Pitt and Humphries[306] compared sutures of 10-0 nylon on a taper pointed needle with similar sutures on a new micro edge taper needle. They found that the new needle caused less damage to the intima, although patency rates were similar for the two needles.

A number of technical aids have been developed to help surgeons achieve maximum accuracy with their suturing. A surgeon working alone or with an unskilled assistant may find Acland's frame clamps[6] useful for holding the long ends of key stay sutures. A surgeon working with an experienced assistant usually prefers to have the assistant apply tension to the key stay sutures so that the

anastomosis is optimally positioned for insertion of the next suture. In cases where the assistant is highly skilled and the anastomosis is not in a difficult location, a lot of time can be saved by using the two surgeon-two needle technique[256] or the two surgeon Tweener manoeuver[57].

The problem of suturing in a confined space can be facilitated by suturing without the use of double approximating clamps[266].

Harashina[138] pointed out that the last one or two sutures were the most critical because the lumen may not be clearly visible through the gap. Usually each suture is tied immediately after placement, but Harashina recommended that it might be advantageous to leave the final two or three sutures untied till suture placement was complete. Foucher[105] has published a modification of the Harashina procedure.

Laser techniques

The word 'laser' is a contraction of **L**ight **A**mplification by **S**timulated **E**mission of **R**adiation.

Lasers are devices for converting light of mixed frequencies into an intense narrow monochromatic beam of coherent light. Typically, a gas-filled tube is illuminated by a light flash to excite its atoms. Light emitted by these atoms is reflected backwards and forwards along the tube and stimulates further emission.

Several different types of laser beam are used in medical practice. The most widely used are the argon laser, the Neodymium:YAG (yttrium-aluminium-garnet) laser, the CO_2 (carbon dioxide) laser, and the thulium-holmium-chromium:YAG (THC:YAG) laser.

Some investigators have attempted to weld blood vessels together using laser beams. The intense monochromatic light of a laser beam produces heat on absorption. Blood vessels are normally transparent to the beam, but when coated with staining substances, they can be converted to black-body absorbers and application of a

tightly focused laser beam converts light energy into heat. A "spot weld" is accomplished by thermally-induced coagulation necrosis at the site of application. This technique has been found to be extremely useful in ophthalmology and a number of investigators have tried using it for microvascular anastomoses. The laser equipment is arranged so that it can be applied while the surgeon is using the operating microscope. The laser beam can be aimed with the assistance of visible laser light such as a helium-neon spot[338].

Jain has investigated the use of the Neodymium:YAG laser[176] for the repair of injuries to small arteries[173], end-to-end microvascular anastomoses[174], and end-to-side anastomoses[175]. He has described the results of a large number of anastomoses in experimental animals and in addition has carried out a number of clinical neurosurgical cases. He reports vascular patency rates in the region of 95% but unfortunately his studies were uncontrolled and are subject to scientific criticism. His observations, however, are interesting. He stated that laser end-to-side anastomosis had advantages:

1. They were fast.
2. There were no sutures to act as foreign bodies and cause needle passage trauma.
3. There was less variance of results due to different skill levels or steadiness of hands because the laser was mechanically manipulated.
4. The technique could be applied to deeper parts of the cranial cavity as well as outer cortex.

Jain used a wavelength of 1064 nanometres because he found that lasers with a wavelength of 1236 nanometres caused endothelial damage and thrombosis. He reported that the technique heated up the media of the vessel wall and, at 70-80^{o}C

produced changes in collagen which fused the two vessels together. His anastomoses withstood intra-arterial pressures of <300 millimetres of mercury. His histological studies indicated that the vascular endothelium regenerated in two or three days, and the fused collagen became fibrotic in about a week.

Bailes et al have also investigated the use of lasers[19]. They tried the Neodymium:YAG laser but quite strongly disagreed with Jain's experimental results[310]. They pointed out that, in their own histological studies, the healing process of laser assisted microvascular anastomoses did not differ from that seen with other techniques, and that there was no reason why it should. They favoured the CO_2 laser for vascular anastomosis and have reported on their extensive experience with this technique[18,312]. Their laser was a low power unit (70 milliwatts) and they used 0.1 second bursts and a spot size of 0.15 millimetres to produce 'spot-welds' between three or four stay sutures. Although patency rates were comparable to sutured anastomoses, they observed a high rate of aneurysm formation in the laser anastomoses. This was attributed to loss of elastic elements at the anastomotic site, and was related to the distance between stay sutures.

Other researchers[106,288,324,330,338,383] have also investigated the use of CO_2 laser assisted microvascular anastomoses. They all used the laser in conjunction with three or four stay sutures. Each group reported that the technique was more rapid than suture technique, and good patency rates were obtained. Aneurysm formation, however, continued to be a problem although the rate varied between studies. Vale et al[394] were particularly concerned about aneurysm formation, and wrote that they could not advocate use of this technique because of the documented vessel wall damage and the lack of simple intra-operative methods for verifying the quality of laser anastomoses.

The argon laser has also been used to effect end-to-end vascular anastomoses[123,309]. The argon laser has the advantage of

emitting visible wavelengths of light, but unfortunately also has the disadvantage that its tissue effect is variable, changing with differences in the visible pigmentation of the tissues.

Despite the optimism of some of the researchers, the use of laser assisted microvascular anastomoses has not been generally adopted. The reason for this is due to a number of factors:

1. The need for expensive equipment.
2. The provision of insufficient initial strength.
3. The high false aneurysm rate.
4. Difficulty in determining just the correct amount of laser energy to apply to the vessel wall to produce a coagulation 'weld' without a damaging amount of mural necrosis.
5. Failure to offer major advantages over suture technique.

Bass et al[24] have recently reported the results of a preliminary study using the THC:YAG laser. They were more optimistic about their results, finding that they were able to make sutureless microvascular anastomoses " .... with excellent histological appearance, 90% short term patency rate and an acceptable initial bursting strength".

Electrocoaptation

High frequency electric currents were introduced into medical practice at the end of the nineteenth century. It was found that frequencies >10,000 cycles/second produced heat rather than muscular contraction and this rapidly led to the use of electrocoagulation for the closure of blood vessels and haemostasis[346].

The technique has been adapted for the repair of blood vessels. The principle behind electrocoaptive microvascular

anastomosis is the ability to produce an adherent and localised coagulum by the passage of high frequency electric current through the adjacent tissues. The current is applied to the anastomotic site with the aid of bipolar electrode forceps.

In 1963, Sigel and Acevedo[345] described the use of electrocoaptation to repair linear incisions in arteries and veins. They also reported the results of experiments in which venous[344] and arterial[346] anastomoses were carried out. Large canine vessels were used in their experiments, and they achieved greater success with venous repairs than with arterial repairs.

Wintermantel[411,412] described a series of microvascular repairs using electrocoaptation technique. He called his technique the Thermic Vascular Anastomosis. A hard but flexible wire was wrapped three times around a cannula to form small wire loops with a constant inner diameter. The diameter was slightly larger than the external diameter of the vessel ends being joined, and the loops were flattened with a finger nail. The loop was then slipped over one of the vessel ends and a cuff was formed with a fine angle tipped forceps. Next the other vessel end was pulled over a second wire loop to form an identical, but opposing, cuff. The wire loops were then coapted and held with microsurgical needle holders. This was the most difficult part of the operation as the two wire loops had to be perfectly congruent in order to permit uniform distribution of the electrical energy. Each wire loop was then connected to an electrical pole and heat was produced with three short applications of a defined high frequency electrical current. After fusion of the two cuffs, the handling ends of the wire loops were cut off with fine scissors.

Wintermantel's results demonstrated that patent anastomoses could be made very rapidly in vessels 0.8 - 1.3 millimetres in diameter. Variation in patency rates between different experimental groups, however, suggested that application of just

the right amount of electrical energy was critical if vessel wall damage, and subsequent failure, was to be avoided.

A sutureless microvascular thermic end-in-end sleeve anastomosis has been described by Duarte et al[100]. They used a bipolar coagulator (emitting 6.5 watts output for arteries and 1.1 watts for veins) to 'spot-weld' the edge of the distal stump which was held in bipolar forceps with a 2.0 millimetre tip. Patency rates of 100% for arteries and 78% for veins were achieved.

The problem with the use of electrocoaptation methods is the difficulty in knowing the correct amount of electrical current necessary to produce just the right amount of coagulation. In the studies described, authors have pointed out that the amount of electrical current used was determined by trial and error using a series of similar animal vessels. In the experimental situation this can be done, but in the clinical situation it is much more difficult because of the variation in vessel size. For this reason, electrocoaptive microsurgical anastomoses will not become clinically feasible until a better understanding of the electrical welding mechanism is available and appropriate equipment is developed.

Mechanical devices

Staplers and couplers have been used both experimentally and clinically for vascular repairs. The devices investigated can be classified into three types:

1. Individual circumferential metallic staples.
2. Everting pinned ring devices.
3. Extra-lumenal cuffs and bushings.

In 1956 Androsov[14] described a stapling device that his medical engineering group had developed in the Soviet Union. Their anastomoses were secured by simultaneously bending

multiple circumferentially placed individual U-shaped staples into a B-shape (in a manner similar to the gastrointestinal stapling devices in contemporary use). Androsov described the technique for end-to-end arterial anastomoses and for use with interpositional reversed vein grafts. Inokuchi[162,163] later described using the device for end-to-side anastomoses. In 1958 Vogelfanger and Beattie[396] described a modified stapler that inserted fine U-shaped tantalum staples. Khodadad[183] used Vogelfanger's stapler in a canine experiment that compared microsurgical anastomoses made with three different types of mechanical couplers. Later, Zingg and Khodadadeh[422] reported on further modifications of the Vogelfanger stapling device.

The use of an everting pinned ring device for end-to-end anastomoses of small arteries was reported by Holt and Lewis[155] in 1960. At about the same time another everting pinned ring device was being developed in Japan by Nakayama, who published a review of previous attempts to use similar devices, and a description of his own technique in 1962[259]. There have subsequently been other reports on the Nakayama pinned ring device[260,290,410] and Nakayama also reported on a modification for venous anastomoses[261]. The technique involves the use of two metal rings which have six evenly placed holes and six pins. A ring is placed over the cut vessel end and the edge of the vessel is everted and hooked onto the pins. Once the other cut vessel is similarly attached to a ring, the two rings are approximated, with the pins of each one placed through the holes in the other, and crimped thus bringing about intima to intima contact and fixation.

A different pinned ring device has been used in Scandinavia and is known as the UNILINK apparatus[291]. This was developed with the idea that it would be better suited than the Nakayama rings for smaller vessels. The UNILINK apparatus uses smaller pinned rings and instruments that have been developed for use in conjunction with the operating microscope. Reports indicate that

anastomoses are successful and the technique is rapid and easy to use[29,33,34,270,292,313].

Obora[273], in an experimental study, described an interesting pinned ring system which used hollow cogwheel-shaped metal devices held together by magnetic power. The anastomoses were described as being simple, rapid and reliable, but no further reports have become available suggesting that the system might not have been as good as it was first thought.

Other types of coupling devices have also been described.

Carter and Roth[53] described a method for anastomosing coronary arteries using polyethylene rings. The use of tantalum cuffing rings for vascular anastomosis was described by both Goetz et al[121] and Urschel and Roth[392] in 1961. Haller et al[133] experimented with the use of grooved tantalum and platinum rings. Weiss and Lam[401] tried using tantalum tubes and tantalum cuffs for bridging vascular defects.

Combinations of metal couplers and cyanoacrylic adhesives were investigated, in the 1960's, by Carton et al[54] and Gottlob and Blümel[125].

Euler et al have reported their results using a teflon cuff secured with a mini-clip[102], and using absorbable cuffs constructed from polylactid (Ethicon)[103]. An absorbable coupling device using vicryl polymer was developed by Daniel[88,89,90] in conjunction with Ethicon.

In general the mechanical devices have not yet been accepted for widespread use. In the laboratory, they have been shown to produce rapid and successful methods for microvascular anastomosis but, in almost all cases, they have been technically difficult to use. The staplers are too large for manipulation under the operating microscope; the pinned ring devices require considerable experience and trained assistance; the coupling devices use excessive vessel length to achieve satisfactory eversion

at the anastomosis, and accurate selection of the correct size of coupler is not easy.

Adhesives

Cyanoacrylics

The properties of cyanoacrylate adhesives have been reviewed by a number of authors[17,56,151,170,240,245,265].

Cyanoacrylate adhesives are formed by the polymerisation of monomers, which can be represented by the chemical formula CH_2=C(CN)COOR. The addition of water, normal saline, or weak bases initiates the reaction that leads to bond formation. Strong bonds are rapidly formed when cyanoacrylics are applied to a variety of different materials. Bonds are chemically stable in the normal tissue environment. It is not necessary to apply heat, pressure, or other technical adjuvants to complete the bond. A variety of cyanoacrylate adhesives are available and are named according to the specific monomer used (e.g. methyl-2-cyanoacrylate, iso-propyl-cyanoacrylate).

Experimental vascular repairs with the cyanoacrylate adhesives[30,54,55,132,170,240,356,402] have tended to be less than satisfactory, with intense inflammatory reactions leading to destruction of adventitia and media and eventual anastomotic failure, although the higher molecular weight homologues have been shown to have less histotoxicity than methyl-2-cyanoacrylate[213,413]. It has been noted that the entry of "glue" into the lumen causes instant thrombosis. Some studies[56,157,340] have reported more favourable results - perhaps this was because they used variations on the sleeve technique, which would decrease the risk of monomer entering the lumen.

The cyanoacrylate adhesives have not become available for general clinical use for a variety of reasons, but in particular because of the intense inflammatory reaction that they generate[127] and because polymeric cyanoacrylate has been shown to cause

fibrosarcomas in laboratory animals[126,327]. The use of this substance has been forbidden by the United States Food and Drug Administration.

Other adhesives

A number of other adhesives have been investigated for possible use in surgery.

Bernhard et al[30] used polyurethane resin for the closure of vascular incisions.

Freeman, who was mainly interested in developing an adhesive neural anastomosis technique, tested a wide range of substances[107,108]. He looked at the use of synthetic lattices, polyurethanes, and tapes (polyurethane, silicone, teflon, and micropore). He concluded that further refinement and continued investigation was warranted, but, as it turns out, none of the synthetic adhesives have survived the test of time.

Fibrinogen adhesive

The use of fibrinogen adhesive is discussed in depth in later chapters. The development of fibrinogen adhesives for use in surgery is described in Chapter 3 (see page 73). A review of the literature concerned with the use of fibrinogen adhesives for microvascular anastomoses can be found in Chapter 4 (see page 96).

Type of anastomosis

End-to-end

The end-to-end anastomosis was the first technique to be used in Microvascular Surgery and it is still the most widely applicable. End-to-end anastomoses are most commonly sutured although they can be made by all the other techniques described above.

An end-to-end anastomosis can be put together in a number of different ways. Ideally the two cut vessel ends are held loosely

together in a double approximating microvascular clamp. Most commonly the anastomosis is then made by initially securing the front wall, then the clamp is rotated, and finally the back wall is secured. It is not uncommon for clinical conditions to be awkward, and the anastomosis has to be made in a deep wound or in limited space. It may not be possible to rotate the clamp, in which case it is necessary to use the posterior-wall-first technique[147,158]. Some find this technique technically more difficult to use, although others use it almost all the time as their technique of choice[232]. An alternate method of making the anastomosis in situations where the clamp cannot be rotated is the side-wall technique[421], a method which we have also found to be very useful for solving this problem.

End-to-side

The end-to-side anastomosis was initially used by peripheral vascular surgeons for inserting saphenous vein bypass grafts in the management of occlusive vascular disease.

The cerebral revascularisation procedures, developed by Yasargil, provided the first requirement for a microsurgical end-to-side anastomosis. In these operations, the superficial temporal artery was anastomosed to a branch of the middle cerebral artery[418,419] but this had to be done in such a way that flow was maintained in the middle cerebral artery.

The end-to-side anastomosis has been important for the revascularisation of free tissue transfers. Ikuta[165] emphasised its importance as early as 1975, and more recently Godina[119] recommended it as his method of choice for the arterial anastomosis in free flap transfers.

Godina[119] wrote that the end-to-side anastomosis had the following advantages:

1. A high success rate.
2. Preservation of all existing vessels in the injured extremity.
3. Greater freedom of operative planning.
4. Technical simplicity in terms of access to the vessels.

The size and shape of the side arteriotomy in the recipient vessel is important. In order to reduce loss of flow, the arteriotomy should be large and elliptical so that the side branch (the donor artery) has a funnel-shaped origin, preferably with a small angle of union[217]. Technically it is not easy to make the side arteriotomy. Pederson and Barwick[301] have recently reported on the use of a vascular punch, but this is still being modified and is not yet available for general use. Acland initially recommended[11] making two scissor cuts each side of a "pick-up" suture. Later he developed the Acland arteriotomy instruments and these work so well that our group no longer finds cutting the arteriotomy to be a problem.

End-to-side branch

This technique is a modification of the end-to-side anastomosis, in which a side branch, adequately sized for a microsurgical anastomosis, is located at, or close to, the selected anastomotic site in the recipient vessels. The donor vessel is anastomosed to the side branch using a conventional end-to-end technique. This method of anastomosis, if by chance it is available, may be preferable to an end-to-side technique, especially if clinical conditions are sub-optimal. I have found it quite useful for the replantation of avulsion amputations of the thumb[38], when we have sometimes anastomosed vein grafted digital arteries to a dorso-radial branch of the radial artery in the anatomical snuff box. I have also used it for venous anastomoses of free tissue transfers in regions where recipient veins have been very large.

End-in-end

The end-in-end intussusception method of vascular anastomosis was first described by Murphy in 1897[99], although it was popularised by Bougle[56] in 1901. It was introduced into microsurgery by Lauritzen[204,208], who described it in his Doctoral Thesis (University of Göteborg, Sweden, 1979), and called it the sleeve anastomosis. Lauritzen found it easier and faster than the conventional sutured anastomosis, pointing out that it required just two sutures. Meier, later in 1978, published a modification of Lauritzen's technique[56]. More recently, Nakayama et al[262] described another modification which produced reliable results in fifteen clinical free flap transfers.

In the sleeve technique the upstream vessel is placed inside the downstream vessel to make an overlap, or sleeve, long enough to prevent leakage. The terms upstream and downstream refer to the direction of flow and may be either proximal or distal anatomically, depending on whether vessels are arterial or venous.

Since Lauritzen's 1978 paper, a number of studies have been carried out to evaluate various aspects of the sleeve anastomosis, and to compare it with the conventional sutured end-to-end anastomosis[66,196,205,252,342,404,405].

The advantages of a sleeve anastomosis over an end-to-end sutured anastomosis are that:

1. It is faster[196,205,342].
2. There is less intimal dissection[209].
3. Aneurysms at the anastomotic site[247] have not been reported.
4. Resistance to irradiation is thought to be improved[198].
5. Patency rates are similar to end-to-end anastomoses[209,342].

The disadvantages of a sleeve anastomosis over an end-to-end sutured anastomosis are that:

1. Intralumenal sutures causing tethering, misalignment and tearing[364].
2. A valve type mechanism may be created at the point of anastomosis[395].
3. Low blood flow may be present for the first few post operative days[404].
4. Surgeons have had difficulty reproducing Lauritzen's patency rates[364].

Despite the enthusiasm of some authors and the excellent results of their experimental studies, the sleeve anastomosis has not become widely adopted. Many, myself included, have found it difficult to reproduce the high patency rates and low complication rates reported by Lauritzen. There is no doubt, however, that others find the technique very satisfactory. Johnston[177] informed me that microvascular surgeons in some parts of China use the sleeve anastomosis almost exclusively. The Chinese technique has been described by Pan and Lee[296].

Cuffing techniques

In the early days of microvascular anastomosis, it was common practice to wrap the anastomosis after it had been sutured. Daniel and Terzis[87] recommended that the material " .... be extremely pliable to avoid cutting into the vessel, sufficiently narrow to fit between the clamps, long enough to be manipulated, and clear enough to permit visualisation of the anastomosis". They recommended a piece of Saran Wrap or clear cellophane. Saran Wrap was also recommended by McLean and Buncke[231], who had also been experimenting with thrombin powder, Gelfoam strips, micropore tapes and silicone sheets.

Hayhurst and O'Brien[150] were enthusiastic about MacLean and Buncke's cuffing technique, noting that it allowed the surgeon to achieve a leak free anastomosis with fewer sutures than would otherwise be necessary. They thought this would diminish the risk of medial necrosis and arterial occlusion. Bell et al[27], on the other hand, were less enthusiastic about cuffed microarteriorraphy for sealing partially sutured end-to-end anastomoses. Their experimental results indicated that cuffed anastomoses (using venous cuffs) were inferior to conventional sutured anastomoses. Hart[148], who was interested in the problem of haematoma formation near anastomoses of vessels containing heparinised blood, reported favourable results after using absorbable gelatin sponge to prevent anastomotic leakage.

Other types of cuffing techniques have also been investigated in the search for more rapid, easier or better anastomoses. Harris et al[146] demonstrated that their autogenous arterial cuff technique needed few sutures, was associated with less vascular trauma, could be completed quickly, and yielded high patency rates. Merrell, Zook and Russell[251] compared four-stitch microarterial anastomoses, reinforced with cuffs of artery, vein or polyglycolic acid, with standard eight-stitch repairs. Nakayama et al[263] experimented with a technique that used a venous cuff and two sutures.

Sanders et al[329] described an interesting anastomotic technique, in which the end of the donor vessel was split longitudinally and wrapped around the recipient arteriotomy to create a sort of cuffed end-to-side anastomosis.

As experience has increased, it has been realised that it is not necessary to prevent anastomotic leakage by wrapping sutured anastomoses. Small leaks rapidly stop by themselves. Large leaks can be stopped by the addition of extra sutures, and these can be inserted as an adventitial layer only, if it is felt that the insertion of additional full thickness sutures might be hazardous[300]. Neither of

these two methods appears to be associated with a decrease in the patency rate. For this reason, anastomotic wrapping is not generally carried out nowadays.

Size discrepancy

The anastomosis of vessels with different diameters poses problems, which can be solved in a number of ways.

Hurwitt et al[160], working with quite large size discrepancies, suggested making variations to end-to-end anastomoses, in which either the large vessel diameter was reduced by suturing or the small vessel diameter was expanded with a fish mouth incision.

For small discrepancies, one technique involves mechanically dilating the smaller vessel, till it reaches a size comparable with the larger vessel, and then carrying out a standard end-to-end anastomosis. Other techniques include the use of an oblique transection of the smaller vessel[142] and, in the case of large size discrepancies, the use of an end-to-side anastomosis.

Harashina et al[141] have described a Y-shaped anastomotic technique for handling venous size discrepancy problems in free flap surgery.

Other important anastomotic factors

A number of specific technical factors, important for anastomotic success, have been discovered, investigated and reported. These have included aspects concerned with microvascular clamps[70,137], preparation of the cut vessel ends[5,11,50,62,87,118,283,335] and the avoidance of tension at the anastomosis[68,99,130].

Other factors that have been investigated have included methods of determining the patency[4,11,50,63,67,81,87,197,283,335] of microvascular anastomoses and studies concerning the use of irrigating solutions[10,234], the effect of hydrostatic dilatation[44,319],

the effect of blood flow[214,362,367,404] and measurements of anastomotic strength[75,92,117,194,311,397].

THE HEALING OF MICROVASCULAR ANASTOMOSES

Patency rate of microvascular anastomoses

Prior to the era of the microvascular anastomosis it was not possible to obtain satisfactory or reliable results in the anastomosis of small blood vessels. Urschel and Roth[393] did manage to achieve a patency rate of 73% in vessels 1.4 to 2.0 millimetres in diameter which was "much higher than expected for vessels of this size and can be attributed in large part to improved methods of anastomotic technique". The improvement in patency rates that occurred with the introduction of microsurgical technique is shown in a table in O'Brien's textbook[283].

The classical paper of Jacobson and Suarez reported a 100% patency rate in vessels 1.4 millimetres in diameter. There was some initial concern about accepting these results and surgeons were uncertain whether they should commit themselves to the expense of purchasing an operating microscope. After Green[128] demonstrated that the results were reproducible, the technique began to be adopted. Soon others[48,71,72,150,164,269,277] began to report patency rates of over 90% in vessels 1.0 millimetre in diameter. These kind of patency rates have subsequently been widely reproduced, and nowadays microsurgeons expect to achieve patency rates in this range for 1.0 millimetre diameter vessels[343].

The patency rate is usually lower for smaller vessels, although skilled surgeons expect to achieve near perfect patency rates for vessels as small as 0.5 millimetres in diameter[1,361]. Fortunately, in clinical practice it is uncommon for vessels to be this small except in very young paediatric patients.

In clinical practice the patency rate also depends on the extent of damage to the vessels[283]. In free tissue transfers, the donor tissue should be no more damaged than laboratory vessels, but the recipient vessels may be damaged by irradiation, dissection through scar tissue, or the effects of injury. In replantations, the extent of damage varies with the type of amputation: guillotine, local crush, or avulsion. The results of replantation in these different types of amputation, and some of the techniques that have been developed to improve microsurgical survival rates, were reviewed in our paper on the replantation of avulsion amputations of the thumb[38].

Histology of microvascular anastomoses

A number of studies have looked at the biological process of healing and regeneration of microvascular anastomoses.

Early studies looked at vessels larger than 1 millimetre in diameter. Histological and histochemical findings, after saphenous veins were grafted into femoral arteries, were reported in a canine model[220]. There was an initial inflammatory response, which was followed by a thickening and fibrosis, with a concomitant increase in ground substance, noted in all layers of the vessel wall. If the vein graft was isolated from ingrowing adventitial blood supply by a nylon cuff, these changes were more severe. These findings complimented those from an earlier study by the same group[416], in which it was demonstrated that venous by-pass grafts obtained their nutrition from intra-lumenal plasmatic diffusion in the first 48 hours, and from adventitial capillary circulation at 72 hours.

Khodadad published three reports[182,184,185] on a study that compared end-to-end anastomoses with longitudinal arterial slits in a feline model. He described the processes of intimal hyperplasia, fibrotic replacement of the media and adventitial thickening in both types of repair. He saw elastic fibres in the new intima, and also noted dilatation of the artery at the level of the circumferential

repair. He found that in some cases the arterial wall decreased to half its normal thickness and thought that this was related to reduction in the media.

The histological features of microvascular repairs in rabbit femoral artery repairs were described by Baxter et al[26] in a paper that was primarily concerned with the histological pattern of different kinds of suturing errors. Like Khodadad they found new elastic fibres forming inside the internal elastic lamina and cellular hyperplasia deep to the intimal layer. They also noted necrosis of the media that had been strangulated by sutures, an observation also made by other investigators[26,43,69,215].

In 1977, the histological features of rat femoral artery anastomoses were described by Acland and Trachtenberg[9], who were interested in the effect of surgical trauma, rather than surgical error. They examined the vessels at various times between 1 hour and 3 weeks, and noted widespread loss of endothelium from clampsite to clampsite, and also extensive necrosis in the media.

The Chinese have also published their view of the pathological changes that take place after anastomosis of small arteries[64].

Blair et al[34] discussed the histological features of anastomoses fixed with mechanical couplers, and described a method of dividing anastomoses into descriptive zones.

Brunner[43] described the histological findings of fibrin-glued microvascular anastomoses.

Feeling that these previous studies had concentrated on the healing that occurred after suture errors or surgical trauma, Lidman and Daniel[215] carried out a study to look at the chronological sequence of healing of an end-to-end anastomosis under optimal conditions. This is the most comprehensive description of the histology of healing microvascular anastomoses. Observations were made at two days, ten days, one month, two months and seven

months and their findings in the different layers of the vessel wall were:

Lumen. At two days there was dilatation at the anastomotic site and this remained throughout the follow up period.

Endothelium. At two days the endothelium was normal between the microvascular clamps except in the immediate anastomotic area where it was absent. There was no mural thrombus, no platelet aggregation and the internal elastic lamina was intact. At ten days an intense repair process was underway. On the lumenal surface there was a single layer of endothelial cells. There was intimal hyperplasia to a thickness that equalled about two thirds of the thickness of the normal media. They were not able to identity the exact nature of these myointimal cells but noted that they had characteristics that resembled the smooth muscle cells of the media with their elongated nuclei, although these were arranged longitudinally rather than transversely. The appearance of these layers did not change appreciably between the tenth day and the end of follow up.

Media and inner adventitia. Hyaline degeneration was seen in this layer in sections taken at both the second and tenth post operative days. The amount of degeneration varied depending on the location of the section and was related to the proximity of sutures. Sections near or under sutures, demonstrated marked loss of cell structure and absence of nuclei. Sections between sutures demonstrated an almost normal histological appearance. Later sections, at one, two and seven months, showed media atrophy, which varied from minimal to complete. If minimal, smooth muscle cells were seen to form a circular arrangement, bordered by an inner and outer elastic lamina. If complete, the media was replaced by intimal hyperplasia.

Adventitia. In a similar manner, the adventitia underwent necrosis if it was trapped under a suture, but otherwise remained

relatively normal in appearance. In necrotic areas, there was loss of nuclei from fibroblasts, although elastin and collagen fibres were still present.

General findings. At two days an early and mild inflammatory response was seen, and was mainly located in the adventitia. The cell population was about 50% polymorphonuclear leukocytes and 50% lymphocytes. Few macrophages were to be seen. By the tenth day, there was an intense inflammatory response and a foreign body reaction around the sutures. Sections demonstrated a tapering of the inflammatory response at one month, with chronic inflammatory cells becoming predominant. At two months the inflammation had almost completely resolved.

Scanning electron microscopy of microvascular anastomoses

The scanning electron microscopic appearance of normal vascular endothelium has been described by Wieslander[408,409]. Studies looking at various aspects of microvascular anastomoses have been reported by other investigators.

Thurston et al[379] examined the effect of microvascular clamp jaw pressure. Histologically, they found that when jaw pressure exceeded $30g/mm^2$ media necrosis resulted and this led to persisting dilatation of the clamped segments. Using scanning electron microscopy, they demonstrated endothelial loss at the clamp sites, regardless of the clamp pressure. The endothelial loss, however, was repaired - in contrast to the permanent media damage.

The effect of needle and suture size has been investigated using scanning electron microscopy by Urbaniak et al[389] and Lourie[219]. As expected, they demonstrated that patency rates were higher when finer needles were used. In addition, they noted that extensive adventitial stripping (3.0 millimetres on each side of a cat femoral artery anastomosis) produced stenosis. Gu et al[129] reported that the site of needle holes and stitches was the main

location of thrombocyte adhesion and aggregation in the normal microvascular anastomosis.

Dirrenberger and Sundt[95] studied endothelial regeneration after canine carotid endarterectomies. They concluded that the endothelium regenerated from intact endothelium adjacent to the injury.

Servant et al[339] found that endothelial regeneration completely covered suture threads in rat aorta anastomoses by four weeks. Other investigators[137,362,382] found that reconstitution of the neo-intima took two weeks, whereas Lidman[216] found the process to be much faster, with complete endothelialisation evident after only one week. Gelderman and Berendsen[114] also found the process to be rapid, finding that the suture line of end-to-side anastomoses became re-endothelialised in four days.

A number of other studies[78,79,167,364,399,400] have examined re-endothelialisation and healing of microvascular anastomoses. The results of these investigations did not differ appreciably from previous studies.

Lauritzen and Hansson[207] looked at the re-endothelialisation of sleeve anastomoses. They reported that the process started before the third day and was complete by the end of the first week.

Nightingale et al[268] have written the most comprehensive description of the scanning electron microscopy of healing microvascular anastomoses, and their findings at different times after anastomosis were:

Zero hours. Sutures could be seen as they perforated the endothelium of the lumen. Suture tension could be observed by looking at the degree of suture flattening. It was rare to see a loose suture, and usually sutures were tight enough to cause bunching of the vessel wall, giving it a corrugated appearance. There was tearing of the endothelium, at the site of suture perforation, and thrombus at both the anastomotic site and at the site of needle

holes. The thrombus was usually a thin layer of platelets, red blood cells and thrombin. At the microvascular clamp sites, there were sharply demarcated areas of endothelial loss, which were also covered with a thin layer of thrombus. Between clamp sites, the endothelium demonstrated loss of surface detail, with scattered areas of desquamation. Some arteries showed sharply defined areas of endothelial loss, which was thought to represent the effect of mechanical dilatation with forceps.

One to twenty-four hours. Anastomotic sites became completely covered with a thin layer of thrombus, which was maximal at four hours. The thrombus consisted of platelets, red blood cells and leukocytes, which appeared from about one hour onwards. Fibrin was rarely present. By the end of the first day the thrombus was gradually diminishing and bare areas were becoming visible on the sutures. After the first hour, the microvascular clamp sites were completely covered with a thin layer of platelets and leukocytes. The surface between the clamp sites was nearly completely covered with a thin layer of platelets, red blood cells and leukocytes. Surface details, such as microvilli and cytoplasmic projections at the junctions of endothelial cells, were not visible. There were scattered patches of endothelial loss, and deficient areas were filled with platelets and platelet debris.

One to seven days. During this period, there was a progressive decline in the amount of thrombus at the anastomotic site, but by one week there was still no evidence of re-endothelialisation. At three days monocytes and/or small macrophages were seen on the sutures. At one week giant cells were common on the sutures and the lumenal surface at the anastomotic site. At the microvascular clamp sites, a sheet of regenerating endothelial cells became visible at about the third day. Along the growing edge individual cells, irregular in outline and orientation, projected outwards from the sheet. It was not clear whether these new cells were laid down over the top of the

thrombus or whether they undermined the thrombotic deposits. Between the clamp sites, the thin thrombus decreased after the first day, and after the third day increasing endothelial cover was observed. The increased visibility of endothelial cells was thought to result partly from loss of thrombus and partly from regeneration.

Two weeks. At this time, regeneration of endothelium was virtually complete. Sutures were almost totally covered and microvascular clamp injuries were no longer visible. The endothelial cells were still irregular in outline and orientation, but by four weeks the cells were axially aligned with well defined margins.

FACTORS RELATING TO ANASTOMOTIC FAILURE

In any project, concerned with the success or failure of microvascular anastomoses, it is important to consider the causes and mechanism of failure. Unfortunately, this is a complex and not completely understood area, and is, itself, a field of intense study at the present time. A review of some of the factors related to microvascular anastomotic failure is indicated but an in-depth discussion is out of the scope of this work.

There are a number of complications that can occur after microvascular anastomosis. The main complications include dehiscence, aneurysm, stricture and thrombosis. Although there has been specific interest in aneurysm formation[247], it is thrombosis that causes most clinical problems.

Every microvascular anastomosis involves an operative procedure that produces the three components of Virchow's Triad which are precursors of intra-lumenal thrombosis:

1. Trauma to the vessel wall.
2. Alteration of blood flow.
3. Changes in blood constituents.

The first stage in the formation of intralumenal thrombosis is the production of a haemostatic platelet plug. It is this platelet plug which causes the immediate intra-operative flow failure which the microvascular surgeon is on his guard to prevent at the time of operation. Tissue damage on the intimal surface of the microvascular anastomosis is a stimulus for the adherence of platelets. Lipoproteins on the surface of the platelets (sometimes called platelet factor 3) catalyse the intrinsic clotting mechanism with the resultant formation of fibrin, which consolidates the platelet plug. Finally the fibrin clot retracts, by an uncertain mechanism that is influenced by the contractile protein complex, thrombosthenin, which is present in platelets.

The clotting mechanism is complex (Figure 1). The objective is to convert the plasma protein fibrinogen into an insoluble network of fibres called fibrin. A common end pathway can be triggered by either an intrinsic system or a faster extrinsic system. The intrinsic system is triggered by contact with an abnormal surface, whilst the extrinsic system is triggered by tissue factors released from damaged tissues. The reactions form a true cascade, for they not only trigger each other in sequence, but the process has the ability to accelerate. Acceleration is brought about by the presence of thrombin, which potentiates the activity of Factors V and VIII as well as causing further platelet aggregation, thus increasing the amount of platelet factor 3.

Once formed, the haemostatic platelet plug may either dissolve, by the process of fibrinolysis, or organise and/or propagate into an intra-vascular thrombosis. Fibrinolysis is important to the success of a microvascular anastomosis, and the process occurs rapidly after surgical manipulation. The mechanism of fibrinolysis

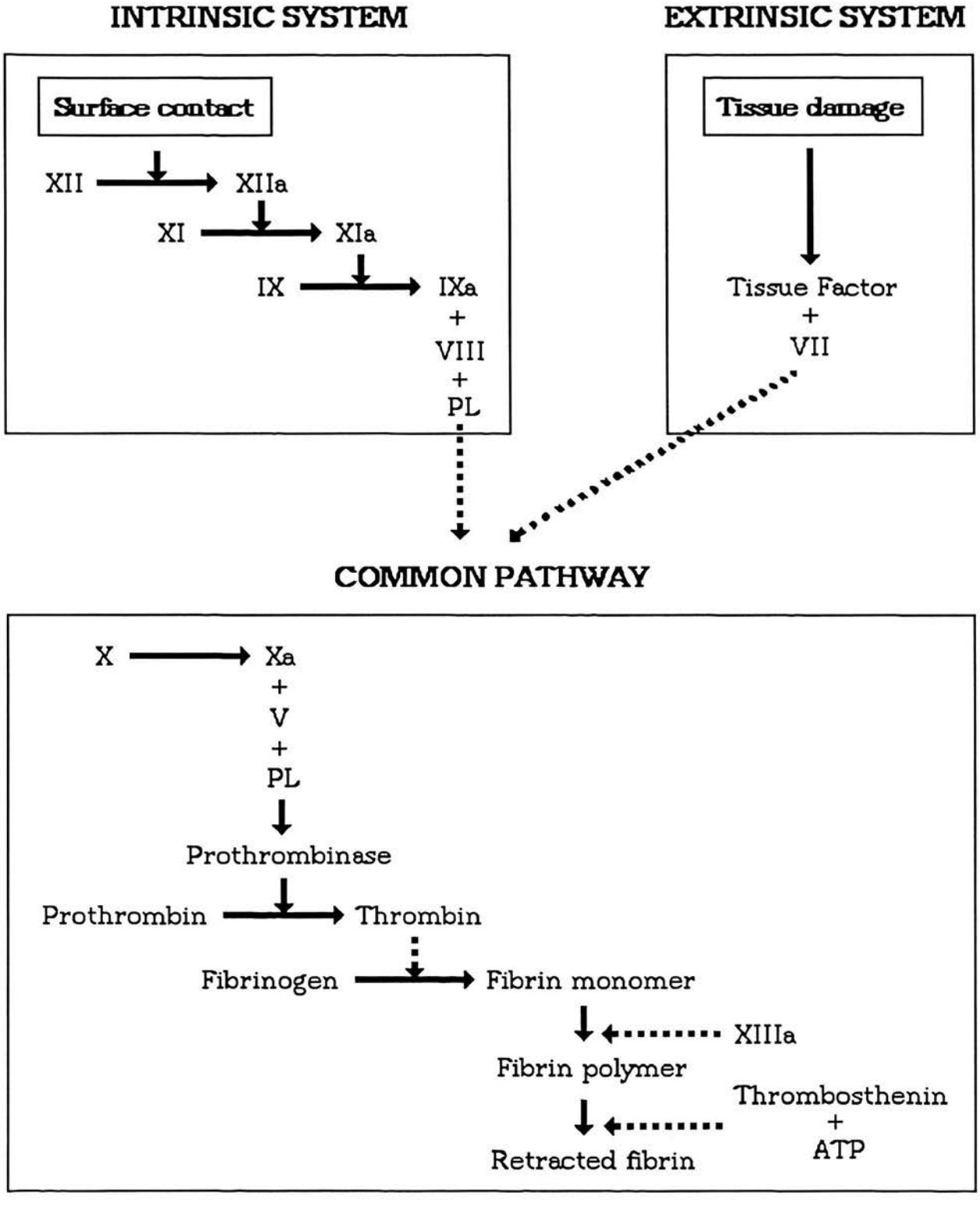

INTRINSIC SYSTEM
EXTRINSIC SYSTEM
Surface contact
XII
XIIa
XI
XIa
IX
IXa
+
VIII
+
PL
Tissue damage
Tissue Factor
+
VII
COMMON PATHWAY
X
Xa
+
V
+
PL
Prothrombinase
Prothrombin
Thrombin
Fibrinogen
Fibrin monomer
XIIIa
Fibrin polymer
Thrombosthenin
+
ATP
Retracted fibrin

Figure 1. A simplified diagram of the blood clotting mechanism.

Solid arrows represent transformations.

Interrupted arrows represent actions.

PL stands for Platelet Factor 3.

A Factor number followed by 'a' indicates the activated form of the Factor.

Ca^{++} is not shown but is required for most steps.

is unaffected by the type of microvascular anastomosis[18]. Platelet plugs are most prominent at about fifteen minutes, and if complete vascular occlusion is going to occur, it usually happens in the first thirty minutes[322].

The problems associated with loss of blood flow through a microvascular anastomosis are complex, particularly when they become associated with increasing ischaemic times in the replanted or transplanted tissue, and the process of ischaemic reperfusion injury starts to evolve. Klitzman[193] has reviewed the factors that determine the perfusion of a given region of tissue, and classified them into geometric factors (active and passive changes in diameter) and rheologic factors (plasma viscosity, platelets, red blood cells, white blood cells).

The basic principles for managing anastomotic failure[181] are:

1. To recognise the problem.
2. To manage the anastomosis.
3. To minimise the ischaemic injury.

The problem is almost invariably at the anastomosis, and this should be surgically corrected, looking carefully for a reason why the clot occurred. Some of the technical factors relating to anastomotic failure have been investigated by Fukui and Tamai[113]. Their experiments documented " .... that:

1. If there is a branch from a damaged artery that constitutes a sufficient collateral route, the success rate of an anastomosis just above the branch is significantly greater higher than just below.

2. When recipient arteries are dissected in preparation for anastomosis, the vessels should have high-pressure outflow to assure a successful outcome.

3. If both recipient and donor arteries are normal and not damaged, anastomosis where turbulence is likely to occur will present no problems."

In the occasional instance, when surgical correction is impossible, pharmacological manipulation of the anastomosis with plasminogen activators, possibly introduced through a side branch catheter, may be beneficial. Clinically, the ischaemic injury[297] can be minimised by cooling and, temporarily (while waiting for operating room availability) with the aid of leeches, an area in which there has recently been much interest[388]. Laboratory experiments, using drugs (such as allopurinol and ibuprofen) to decrease free radical production, and drugs (such as superoxidase dismutase and deferoximine) to scavenge free radicals, are providing promising results and, in the future, may be added to the clinical armamentarium.

CHAPTER 3

Fibrinogen Adhesive and its Use in Surgery

INTRODUCTION

In the first part of Chapter 2 many different approaches to the technique of microvascular anastomosis were described. One of these involved the use of adhesives. Unfortunately, the synthetic adhesives proved to be unsatisfactory for anastomoses, not so much for technical reasons, but rather because of their detrimental effect on the surrounding soft tissues.

Chapter 3 begins by considering features thought to be desirable in an ideal adhesive. Next there is a review of the historical milestones in the development of fibrinogen adhesives for surgery. This is followed by the main thrust of the chapter, which is a detailed description of Tisseel, a current commercially available fibrinogen adhesive.

THE IDEAL SURGICAL ADHESIVE

Daniel and Terzis[86] have reviewed the qualities that they consider to be desirable in an ideal adhesive substance for surgical application. They suggest that it should:

1. Adhere and set quickly without excess heat production.
2. Remain stable at body temperature.
3. Work in a moist environment.
4. Be noncarcinogenic and nondetrimental to healing.
5. Be sterilisable.

According to these criteria, fibrinogen adhesive would appear to be a very satisfactory material, but in order to determine if it can be satisfactorily used for making microvascular anastomoses, it will

be necessary to investigate and answer a number of questions about its use:

1. Can a fibrinogen adhesive anastomosis produce statistically comparable patency rates to a conventional suture technique?
2. What are the morphological changes that occur at anastomotic sites?
3. What advantages does an fibrinogen adhesive anastomosis have compared to a conventional sutured anastomosis?

This chapter has been devoted to describing the development, mode of action and application of a fibrinogen adhesive. The following chapters describe experimental work carried out to answer the three questions above.

THE DEVELOPMENT OF FIBRINOGEN ADHESIVE

The idea of using clotting constituents from human blood as an adjunct to surgery dates back to the beginning of the 20th century. Initially they were used to help obtain haemostasis, but subsequently they have been used for many other purposes.

The historical milestones, and original references concerning the development of fibrinogen adhesive, have been recorded by Schlag and Redl[332]. In 1909 Bergel described the haemostatic effect of fibrin powder. Later, Grey in 1915 and Harvey in 1916, both in the United States, used either fibrin or fibrin patches to arrest haemorrhage in the brain and other viscera. The concept was then lost and was not rediscovered till the 1940s.

In 1940, Tarlov and Benjamin[370], in the United States and Young and Medewar[420], in the Great Britain, published the results

of experimental nerve anastomoses in which they used a fibrin adhesive to maintain nerve stump approximation. Results did not meet their expectations due to poor strength and stability in the adhesive. They suggested that the lack of adhesiveness and stability was related to an inadequate concentration of fibrinogen and the absence of antifibrinolytic substances. It must also be remembered that Factor XIII was unknown to them (Laki and Lorand discovered Factor XIII in the late 1940s).

Also in the 1940s, Tidrick and Warner, were working with thrombin as a haemostatic agent[380] and for the fixation of skin transplants[381].

Work continued with other types of resins and adhesives through to the 1970s.

In 1972, in the German literature, Matras et al published the successful results of a peripheral nerve repair experiment in which they used a cryoprecipitate of concentrated fibrinogen which contained an enhanced content of Factor XIII. Problems encountered were associated with fibrinolysis. Aprotinin, isolated by Kunitz and Northrop, was incorporated to inhibit fibrinolysis and delay the onset of fibrin degradation.

In 1975, the first successful application of fibrin adhesive for human nerve repair was published, in the German literature, by Matras and Kuderna.

Matras[244] reported that the first recorded use of fibrinogen adhesive for microvascular surgery was recorded in the German literature in 1977[243]. Karl, Tilgner and Heiner[179] reported that soon afterwards Dinges et al[94] recorded, in the German literature, the histological healing process of experimental anastomoses using a combination of fibrinogen adhesives and sutures. Matras[244] reported that it was not long before a clinical series of satisfactory neurosurgical arterial microvascular anastomoses was reported in the German literature[191].

It is impossible to separate a discussion on fibrinogen adhesive from the process of wound repair, in which endogenous fibrin plays an important part. For this reason this section will commence with a review of the process of wound repair.

The healing of traumatic wounds

The process of wound healing involves two main components that are integrally related:

Contraction. This is the mechanical reduction in the size of the wound which takes place during the first few weeks. The process is probably mainly brought about by granulation tissue through the action of myofibroblasts, although the contraction of collagen fibres and the removal of fluid by drying have also been suggested as contributory factors.

Repair. This is the replacement of lost tissue by granulation tissue which matures to form scar.

To facilitate understanding, the process can be divided into three phases, although this is purely artificial and there is considerable overlap between them:

1. Traumatic inflammation. In this phase, tissue trauma is immediately followed by coagulation and haemostasis. The blood clotting process produces fibrin, which is also a product of the acute inflammatory response initiated by cellular damage. The inflammatory response starts within hours and extends to about the fourth or fifth day. Fibrin brings about recruitment in the injured tissue, activates macrophages and chemotactically attracts polymorphonuclear leukocytes. These polymorphonuclear leukocytes, which are also attracted by plasma components and

chemotactic substances released from aggregated platelets, are quickly subject to lysis and their main task is to degrade damaged tissue and to phagocytose cellular debris.

2. *Demolition.* In this phase, lysosomal enzymes, either released from dead tissue cells or from disintegrating polymorphonuclear leukocytes, bring about a debridement of the wound. Monocytes migrate into the wound and change into macrophages, becoming maximal in number between the fourth and fifth days. Macrophages, which may fuse to form foreign-body giant cells, have several functions. These include regulation of coagulation and fibrinolysis; the removal of tissue debris, cells and bacteria; and the release of growth factors which stimulate angiogenesis and myofibroblast activity.

3. *Ingrowth of granulation tissue.* This phase also gets underway during the first week. Macrophages invade the fibrin clot, advance towards the centre and are followed closely by a vascular granulation tissue consisting of capillary loops and myofibroblasts. The insoluble fibrin clot is replaced in a process called organisation. Macrophage growth factors stimulate the production of granulation tissue, although platelet-derived growth factor is also important for the attraction and activation of myofibroblasts. Differentiation soon occurs. Arterioles and venules form within the vascular network, and the large plump 'fibroblast precursors' develop the characteristic features of myofibroblasts. The latter secrete soluble collagen, which polymerises to form fibrils, and mucopolysaccharide ground substance. Later, in this phase, as maturation proceeds, nerve fibers and lymphatics form, and mature collagen fibres are seen, orientated according to the stresses applied to the wound. Advancing maturity is associated with devascularisation, some vessels undergoing atrophy whilst others show endarteritis obliterans. This results in the formation of a pale avascular scar and may be associated with local tissue distortion in a process known as cicatrisation.

Basic principles behind fibrinogen adhesive

The above review indicates that naturally occurring fibrin is an important substance in the process of wound healing. Fibrin acts as a sealant, preventing the loss of body fluids; it forms a barrier against external contamination; and it provides a substrate material for the ingrowth of reparative granulation tissue.

Naturally occurring fibrin is formed in the final common pathway of the blood clotting mechanism (Figure 1). Fibrinogen is converted to fibrin by the action of thrombin, which splits off two peptide fragments called fibrinopeptides A and B (see page 83). The remainder of the fibrinogen molecule undergoes spontaneous polymerisation with its fellows. In this manner, the fibrin monomer forms orderly molecular aggregates which build up to a network of fibres that become known as a clot. Factor XIII, fibrin-stabilising factor, creates covalent bonds between the aggregates, thus converting the easily disrupted soluble fibrin into stable insoluble fibrin and the clot becomes a firm gel.

Fibrin clots can be similarly created in vitro using products obtained from donated blood through blood transfusion services. This forms the basis for the production of fibrinogen adhesives.

Fibrinogen adhesives have three effects that have been put to use in surgical procedures:

1. *Haemostasis.* Fibrinogen adhesives have been used to obtain haemostasis in situations where there is blood loss from wide surfaces but no specific vessels for ligation or cauterisation. The achievement of haemostasis avoids the problem of haematoma formation in the wound. This is an advantage because the lengthy process of absorption and/or organisation of the haematoma is not necessary, and because haematoma has a rather negative influence on the quality of granulation tissue formed.

2. *Adhesive.* Fibrinogen adhesives have, as their name implies, been used to hold structures together. Critics have

repeatedly pointed out that their adhesive strength is quite limited and will not tolerate exposure to major stress. Fibrinogen adhesives, however, are not used to obtain adherence of major tissue segments. Rather, they are used to maintain approximation of adjacent tissue surfaces which are not under external stress. They do not introduce an artificial barrier (like the synthetic adhesives), but form a tight biological seal (keeping out haematoma and other adjacent tissues) and, in this way, aid in smooth healing of the 'glued' structures.

3. *Fibrin network.* The fibrin network is a good substrate for the ingrowth of granulation tissue and particularly for the attraction of fibroblasts. This effect is thought to be beneficial for wound healing[357], and has been used beneficially for the healing of ulcers unresponsive to other forms of management[331].

Fate of fibrinogen adhesive in the tissues

Fibrinogen and fibrin are removed from the tissues by the process of fibrinolysis, which is brought about by proteolytic enzymes.

Intra-vascularly, the fibrinolytic enzyme, plasmin, is produced by the activation of circulating plasminogen. This can be initiated in a number of ways. Prekallikrein is the inert precursor form of the enzyme kallikrein, which is present in concentrated form in the lysosomes of most tissue cells, and has also been isolated from leukocytes and platelets. Plasminogen activators have been identified with kallikrein. Plasmin has a wide range of activity. In addition to degrading fibrin and fibrinogen, it also degrades other clotting factors and a variety of plasma proteins such as complement and some hormones. Kallikrein is released in vivo by vasoactive stimuli such as physical stress, histamine, adrenalin, hypoxia, ischaemia and bacterial pyrogens. It is presumed that, in these circumstances, prekallikrein is released from the endothelial cells of small blood vessels, although it is known that prekallikrein

can also be released from the lysosymes of other cells both physiologically and in circumstances where there is shock or tissue damage. Activation of plasminogen occurs on the fibrin rather than in the blood itself. Plasma proteins that specifically neutralise free plasmin also exist. The concentration of plasmin in the blood varies and, thus, there appears to be a delicate balance between the two extremes of intravascular clotting and afibrinogenaemia.

When large deposits of fibrin exist extra-vascularly, either as haematomata, as inflammatory exudates or after the application of a fibrinogen adhesive, they are found to be quite stable. They are removed during the demolition phase of wound healing. Digestion is brought about by proteolytic lysosomal enzymes derived from invading inflammatory cells and also by plasmin derived from the the activation of plasminogen by kallikrein in the endothelium in the advancing granulation capillaries. The fibrin is broken down to fibrin degradation products and is then replaced by granulation tissue. The rate of degradation is dependent on the fibrinolytic activity of the surrounding tissues, and, in the case of fibrinogen adhesives, on the amount of added antifibrinolytic substances added. Large quantities of fibrin slow down cell migration and thus wound healing becomes delayed.

COMMERCIALLY AVAILABLE FIBRINOGEN ADHESIVE

Fibrinogen adhesive is produced both commercially and non-commercially.

The Österreichisches Institut für Haemoderivate, a subsidiary of Immuno AG in Vienna, has developed a commercial fibrin sealant (note that the terms fibrinogen adhesive and fibrin sealant refer to the use of the product rather than its content and are, therefore, mutually interchangable) and this is readily available (note that, in the United States, Tisseel is still pending Food and Drug

Administration approval) and licensed under the trademarks Tissucol, Tisseel and Fibrin-kleber Human Immuno.

This fibrinogen adhesive is commercially available in Canada and licensed under the trademark Tisseel. It is marketed by Immuno (Canada) Ltd, 5334 Yonge Street, Suite 1727, Toronto, Ontario.

Packaging and composition

A Tisseel Kit (Figure 2) contains five separate vials containing substances which, when mixed correctly, form a two component adhesive, of which fibrinogen is the main active ingredient. The contents of the vials are listed in Table 1. Of the five vials, only four are used for a given application because two different concentrations of thrombin are provided in each Tisseel Kit. This allows a choice of two clotting rates.

The protein concentrate is made from pooled fresh frozen human plasma, whereas the aprotinin and thrombin is bovine in origin.

Each Tisseel Kit also contains an application set consisting of a Duploject applicator, disposable syringes, two joining pieces and four application needles.

Four types of pack are available, each containing a different quantity of Tisseel. Tisseel Kits 0.5, 1.0, 2.0 and 5.0 each provide either 0.5, 1.0, 2.0 or 5.0 millilitres of reconstituted Tisseel solution and a matching amount of Thrombin solution.

The shelf life of Tisseel is twenty-four months when stored between +2^{o}C and +8^{o}C. It is advised that reconstituted Tisseel and thrombin solutions are used within four hours.

Mode of action

The fibrinogen adhesive, Tisseel, acts by converting fibrinogen to fibrin under the influence of thrombin. It is an imitation of the final sequence of blood clotting, although the


A
Two-Component Fibrin Sealant
Tisseel™ Kit 0.5

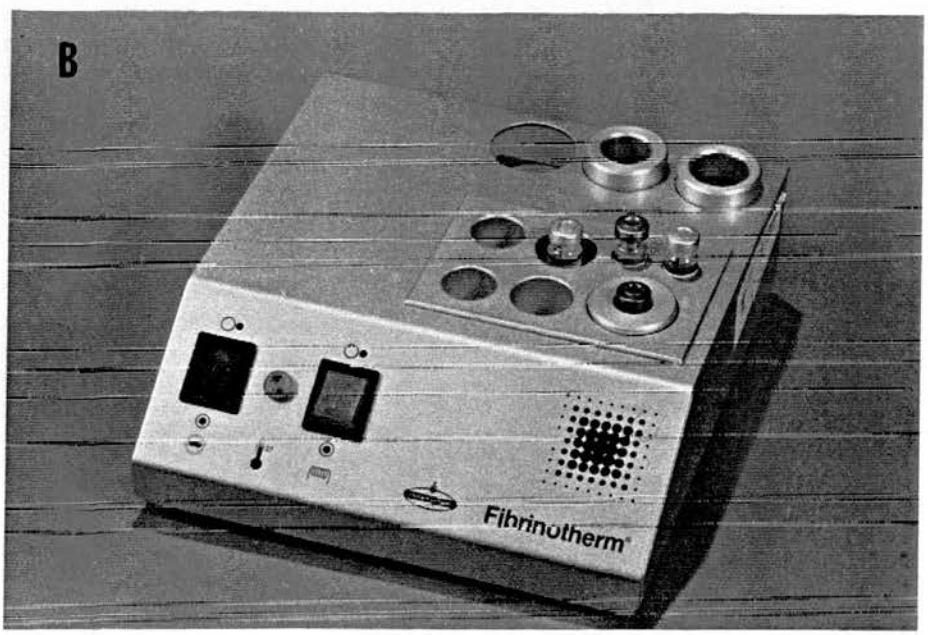

B
Fibrinotherm®

Figure 2. The commercially available fibrinogen adhesive used in the preliminary and experimental studies.

A The Tisseel Kit 0.5, illustrating the outer packaging, the five vials used to make up the two components and the Duploject system for application.

B The custom designed Fibrinotherm heating and stirring device.

TABLE 1. Contents of the five vials contained in each Tisseel Kit.

Protein Vial.

Tisseel protein concentrate (human), freeze dried and heat treated. (Contains a magnetic bar to facilitate reconstitution when put into the Fibrinotherm warming and stirring device).

Reconstituted contains:

Fibrinogen	70-110 mg/ml
Fibronectin	2-9 mg/ml
Factor XIII	10-50 U*
Plasminogen	40-120 µg/ml
Human albumin	20-50 mg/ml
TOTAL PROTEIN	100-160 mg/ml

Aprotinin Vial.

Aprotinin solution (bovine).	3000 KIU/ml†

Thrombin 4 Vial.

Thrombin 4 (bovine), freeze dried.

Reconstituted contains:	4 IU/ml‡

Thrombin 500 Vial.

Thrombin 500 (bovine), freeze dried.

Reconstituted contains:	500 IU/ml‡

Calcium Chloride Vial.

Calcium chloride solution.	40 mmol $CaCl_2$/l

* One unit corresponds to the amount of Factor XIII contained in 1 ml of fresh normal plasma.

† 30 Kallidinogenase Inactivator Units (KIU) correspond to 1 FIP unit.

‡ One International Unit (IU) of Thrombin is defined as the activity contained in 0.0853 mg of the First International Standard of Human Thrombin.

concentration of fibrinogen is very high in the product to compensate for the absence of platelets.

A detailed plan of the sequence of events that takes place is illustrated in Figure 3.

The first component consists of a vial containing freeze dried and heat treated protein (mainly fibrinogen; other plasma proteins such as fibronectin [cold insoluble protein] and albumin; and Factor XIII) mixed with a vial containing aprotinin solution.

The second component consists of a vial containing freeze dried thrombin (a choice of two different concentrations is provided) mixed with a vial containing calcium chloride solution.

The preparation of Tisseel fibrinogen adhesive is diagrammatically illustrated in Figure 4.

When the two components are mixed, thrombin converts fibrinogen into activated fibrin monomers. This initiates clotting and the mixture solidifies. The pharmacodynamics of the process have been described by Redl, Schlag and Dinges[314].

Fibrinogen (Figure 5) is a protein with a molecular weight of about 340,000. The molecule consists of six polypeptide chains of three different types: alpha, beta and gamma. The chains are all connected through disulphide bridges. Thrombin splits the fibrinopeptides A and B from the fibrinogen molecule leaving fibrin monomer. Hydrogen bonding produces aggregation of the fibrin monomers and this produces the fibrin clot.

The thrombin simultaneously activates Factor XIII to the transaminase Factor XIIIa in the presence of calcium ions.

Factor XIIIa acts to immediately (3 to 5 minutes) cross-link the fibrin gamma chain to the gamma-gamma dimer. The beta chain does not react and alpha chains are cross-linked by peptide-like bridges to an alpha polymer.

Factor XIIIa also acts as a catalyst for the formation of fibrin cross-linkages with collagen. The plasma protein fibronectin plays

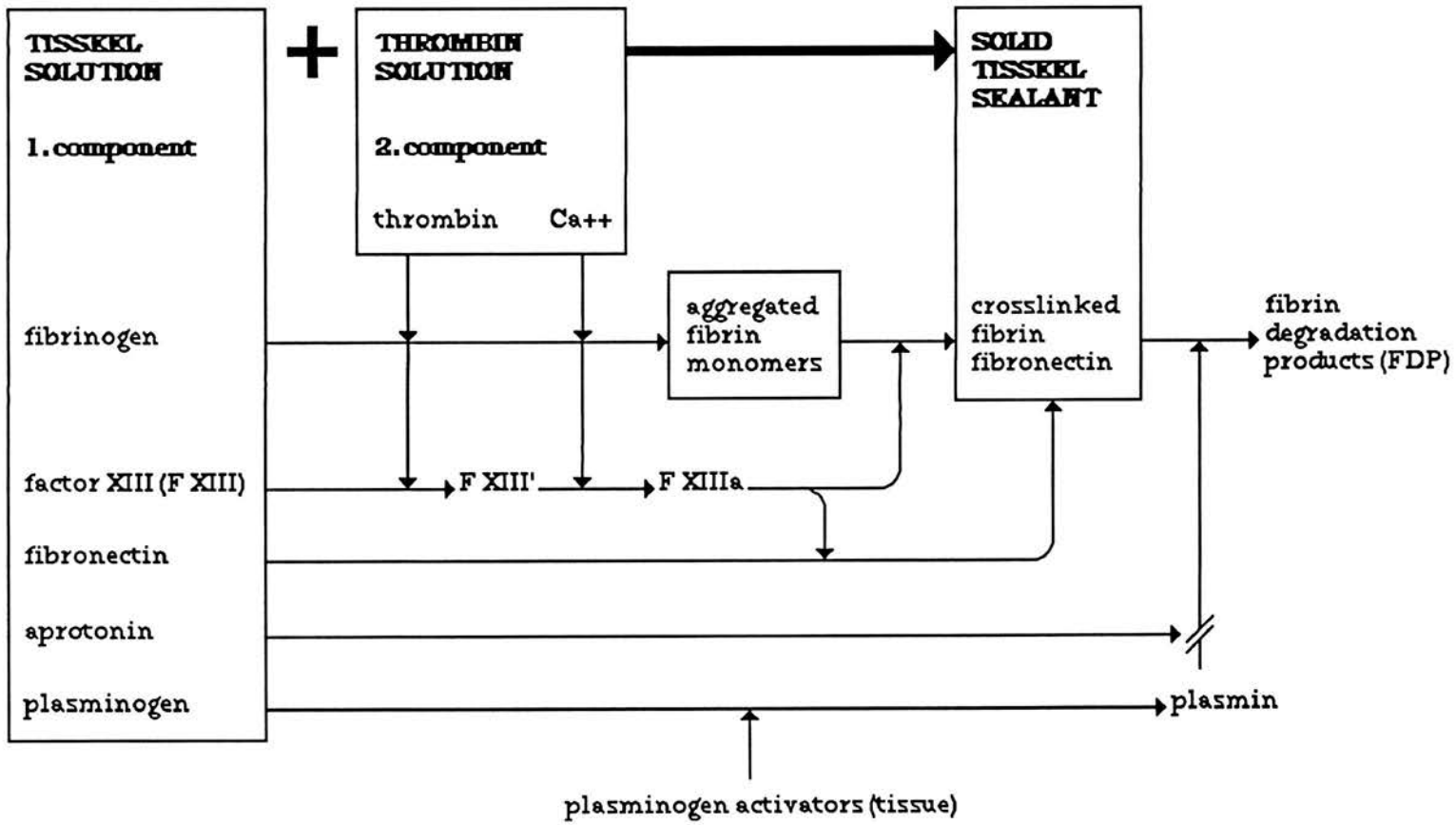

TISSEEL
SOLUTION
1. component
fibrinogen
factor XIII (F XIII)
fibronectin
aprotonin
plasminogen
THROMBIN
SOLUTION
2. component
thrombin
Ca++
aggregated
fibrin
monomers
F XIII'
F XIIIa
SOLID
TISSEEL
SEALANT
crosslinked
fibrin
fibronectin
fibrin
degradation
products (FDP)
plasmin
plasminogen activators (tissue)

Figure 3. Diagrammatic representation of the mode of action of Tisseel.

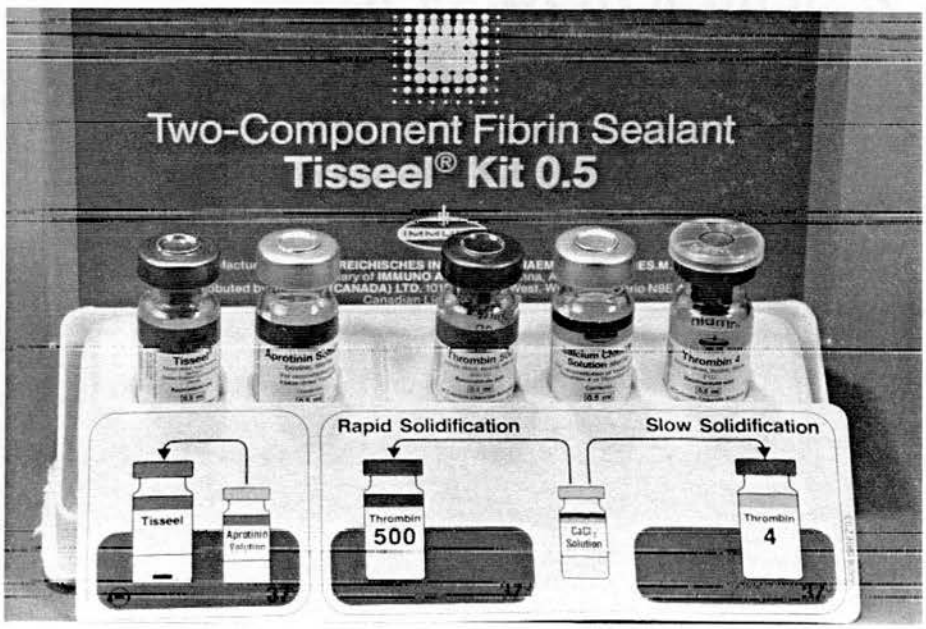

Two-Component Fibrin Sealant
Tisseel® Kit 0.5
Rapid Solidification
Slow Solidification
Tisseel
Thrombin
500
Thrombin
4

Figure 4. A Tisseel Kit 0.5 with its enclosed diagram illustrating the method of preparation of the two components of the fibrinogen adhesive using material from four of the five vials.

Note that only one of the two vials of thrombin is used, depending on whether rapid solidification (thrombin 500) or slow solidification (thrombin 4) is required. The adhesive microvascular anastomosis described in this study used thrombin 500.

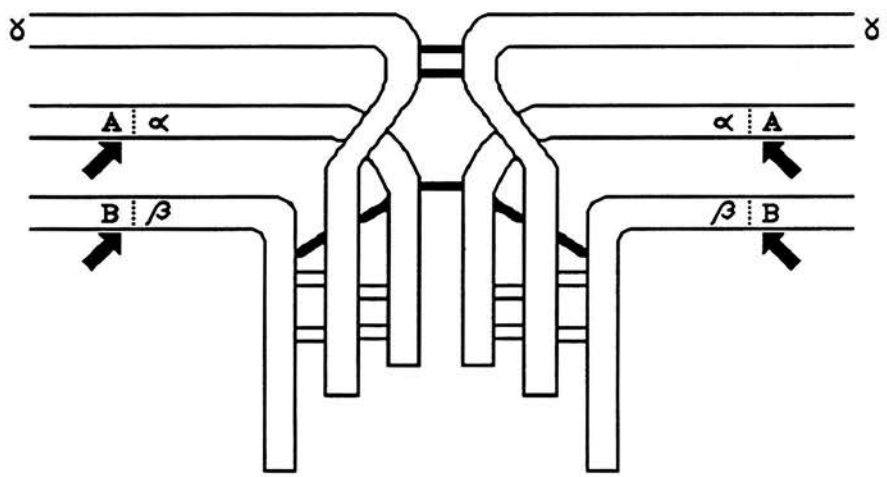

γ
γ
A
α
α
A
B
β
β
B

Figure 5. Diagrammatic representation of the fibrinogen molecule demonstrating the six polypeptide chains (three different types: alpha, beta and gamma) and the interconnecting disulphide bridges.

The arrows indicate the locations in which thrombin splits the fibrinopeptides A and B from the fibrinogen molecule leaving fibrin monomer.

an important role in this process, which results in an increase in the clot's strength.

The addition of aprotinin to the fibrinogen adhesive retards the inevitable process of fibrinolysis and absorption of the clot during the process of wound healing.

Developmental improvements. As experience was gained, changes were made with the developing fibrinogen adhesive in order to bring about product improvement.

Fibrinogen. Initial attempts at making fibrinogen adhesives, using autologous material, were unsatisfactory because of poor tensile strength in the clots. Production method refinements for the fibrinogen component, including the pooling of fresh frozen human plasma, have resulted in an increase in the concentration of fibrinogen. Modern product contains between 100 and 160 mg/ml of total protein, of which about 70 to 80% consists of fibrinogen. A further improvement has been the lyophilisation of liquid fibrinogen without any alteration of its properties. This has produced a product which is much easier to handle and transport.

Thrombin and calcium chloride. Initially, a bovine thrombin in a concentration of 500 IU/ml dissolved in 12 mmol $CaCl_2$ was used. It was found that the clot formation was too rapid and the solidification was less than satisfactory. Modifications have been made to the concentrations of thrombin and $CaCl_2$ to correct these factors.

Antifibrinolytics. One of the advantages of fibrinogen adhesive is its ability to be resorbed, but this must occur in a controlled manner. The process of fibrinolysis is not well understood and varies from tissue to tissue. The process is determined by plasminogen activators which are mainly localised in endothelial cells. Highly vascularised tissues such as lung and kidney have high fibrinolytic activity. Aprotinin is one of several substances that were investigated for their antifibrinolytic action. It is added to the

adhesive to retard fibrinolysis. The dose provided is 3000 KIU/ml, although other concentrations are also available.

Factor XIII and fibronectin. There is experimental evidence that collagen and fibronectin are cross linked with fibrin in the presence of Factor XIIIa. This may explain some of the adhesive qualities of Tisseel. The role of fibronectin is poorly understood, but it has been found on the surface of fibroblasts. Tisseel contains three times as much fibronectin as normal plasma.

Mixing

The substances in the Tisseel Kit are mixed to prepare two components (Tisseel sealant and thrombin/calcium chloride) which are then mixed at the time of application, rather like an epoxy cement (Figure 4).

The four vials containing protein concentrate, aprotinin, calcium chloride and the selected concentration of thrombin (thrombin 500 for rapid solidification or thrombin 4 for slow solidification) are initially warmed to 37°C. This is carried out by placing them in a custom designed heating and stirring device known as Fibrinotherm (Figure 2). Fibrinotherm is a thermablock with a magnetic stirrer arranged to rotate a metal bar in the protein concentrate vial. The vials reach 37°C in ten minutes.

Colour coded syringes are then used to transfer the aprotinin solution to the lyophilised Tisseel vial and the calcium chloride solution to the selected thrombin vial. The vial containing Tisseel sealant is then stirred in the magnetic stirrer until clear, a process that normally takes about five to ten minutes.

The time taken for preparation and mixing does not delay the surgical procedure because it is carried out in an unsterile field by the circulating nursing staff in the operating room. Once reconstituted, the vials containing each of the two components are kept warm in the Fibrinotherm until they are needed for application.

Application

For transfer to the sterile field, the circulating nurse holds the sterile vials and the scrubbed nurse withdraws the two component solutions, again using colour coded syringes.

The two components of fibrinogen adhesive can be applied in a variety of ways. They can be applied sequentially, premixed or simultaneously mixed from a double-syringe applicator known as a Duploject (Figure 2). The Duploject system is widely used because it is versatile, offering the advantages of single-handed operation, thorough mixing and thin-layer application.

The adhesive can be delivered through needles, spray heads or catheters according to specific need for the application. It is possible to use the spraying catheter through the biopsy canal of an endoscope and, in addition, special micro-application techniques are possible.

For optimal benefits, users should adhere to a number of technical details during application:

1. Tisseel should be fully mixed, dissolved and warmed to 37°C.
2. The recipient surface should be as dry as possible.
3. The thrombin concentration should be adjusted for the required clotting time. Thrombin 4 IU/ml produces slow solidification and takes about 30-60 secs. Thrombin 500 IU/ml produces rapid solidification and takes less than one second.
4. Tisseel is best applied as a thin film.
5. Mechanical stress should be avoided for at least three minutes after application.

Once the two components are mixed, they form a viscous solution with strong adhesive properties. This adheres to the

tissues and then solidifies to form a white rubbery mass which gains strength for two hours following application.

Combination with other materials

Biomaterials, such as Dacron patches, lyophilised dura, fascia, muscle, vascular grafts, decalcified bone and hydroxyapatite, have been used in conjunction with Tisseel to supplement its function or to provide additional mechanical support[316]. Experiments testing the interaction of Tisseel with a variety of collagen fleeces have been carried out by Redl and Schlag[316].

Antibiotics have been used in combination with Tisseel both experimentally and clinically. The German literature has been summarised by Redl and Schlag[316]. Drug release from the fibrin clot is probably by simple diffusion and thus depends on the concentration gradient between the clot and the surrounding tissues. It is unlikely that adequate local drug concentrations are maintained for more than three days. It is advised that the total dose of drug used should be less than the recommended maximal daily systemic dose.

Tisseel does not have any known adverse interactions with other substances.

Side effects and contraindications

There are no known specific side effects or contraindications for the use of Tisseel. For obvious reasons Tisseel must not be administered intra-vascularly. Alcohol and iodine may denature Tisseel and these chemicals should be removed prior to the application of the adhesive.

There are no recorded cases of hypersensitivity to Tisseel, and in none of the numerous experimental studies that have been undertaken with the material, has there been any evidence of an unfavourable immunological response.

Tisseel is manufactured from pooled plasma and the potential problems arising from this are discussed in the following section entitled 'Risk of disease transmission'.

Risk of disease transmission

As the Tisseel protein concentrate is made from pooled fresh frozen human plasma there is concern that it is possible to transmit disease with its use. Numerous safeguards are incorporated in the production process.

Plasma donors are tested for HB_S-antigenaemia, anti-HTLV III, and alanine aminotransferase (ALT) levels each time they donate. Positive donors are irrevocably excluded from the plasmapheresis program. In addition Tisseel, once made, is subjected to a product specific heat treatment, which has been demonstrated to produce a decrease in HTLV III titre of 10^4 or more infectious units per millilitre.

A randomised controlled study on the risk of hepatitis transmission using Tisseel has been undertaken and preliminary results are available[101]. None of the patients contracted hepatitis B or non-A/non-B hepatitis. Although one can always argue that the study group was not large enough and the follow up was not long enough, it is a fact that there are no known cases of hepatitis which have been caused by the surgical use of fibrinogen adhesive.

The risk of transmission of other viral diseases, and in particular the risk of transmission of the Adult Immune Deficiency Syndrome (AIDS) virus appears to be either extremely low or non existent. There are no known clinical cases, despite the widespread use of fibrinogen adhesives in the years before stringent precautions were taken with blood products and in areas where AIDS was endemic. It is generally believed that the mechanism of transmission for the AIDS virus parallels that of the hepatitis virus and thus the above mentioned study[101] is reassuring. In a randomised clinical trial, in which Tisseel was used on 333

patients undergoing re-sternotomy or re-operation after cardiac operations, in the United States[323], there were no adverse reactions, no transmission of hepatitis B or non-A/non-B hepatitis, and no human immunodeficiency virus seroconversion. This is the only study that has specifically looked at the incidence of human immunodeficiency virus seroconversion in patients that have been exposed to fibrinogen adhesive. It is presumed that the stringent testing of plasma donors now in place will completely eradicate the possibility of AIDS virus transmission through the use of Tisseel.

Detection in the tissues

At the time of application, it is easy to see clotted Tisseel in the tissues because of its opaque white appearance. If it is necessary to observe the unclotted solution as it is being delivered, disulphine blue dye can be added[316].

For radiographic detection it is possible to add contrast media[316]. Metrizamide is probably the best available but unfortunately it causes some depression of fibrin alpha chain cross linking.

Histologically Tisseel is easily seen using haematoxylin and eosin stain or trichrome stain. Differentiation between Tisseel and endogenous fibrin requires some experience. The difference is that Tisseel does not react as well as endogenous fibrin, perhaps due to the thicker network of fibrin strands in Tisseel. Immunoperoxidase techniques and Mallory's phosphotungstic method have both proved to be useful in animal experiments[316].

Cost

At the time of writing the current cost of a 0.5 millilitre Tisseel Kit 1.0 is $50.00 CAD. There is no capital cost outlay. The Fibrinotherm is provided on loan by the company to Canadian institutions using Tisseel sealant.

This cost can be compared to the cost of microsurgical sutures. At the present time one Davis and Geck 10-0 dermalon microsuture on a 100 micron needle costs $21.07 CAD, and one Ethicon 10-0 ethilon microsuture on a 100 micron needle costs $21.20 CAD.

SURGICAL APPLICATION OF FIBRIN SEALANT

Fibrin sealant has three qualities that have been used in surgery:

1. Haemostasis.
2. Adhesiveness.
3. Improved wound healing.

Surgeons in various different specialties have used fibrin sealant for many different purposes. Almost all the papers are published in the German literature but Redl, Schlag and Dinges[314] have summarised much of their content.

In *abdominal surgery*, fibrin sealant has been used for haemostasis, particularly in cases of rupture of the liver or spleen. It has also been used in the surgery of pancreatic fistulas, and as reinforcement for sutured gastric and colonic anastomoses.

In *thoracic surgery*, fibrin sealant has been used in the management of idiopathic pneumothorax and for making airtight seals after partial pneumonectomy.

In *cardiovascular surgery*, fibrin sealant has been used for sealing Dacron grafts and for a number of different functions during aorto-coronary by-pass surgery, where it is particularly useful when the patient is on extracorporeal circulation.

In *plastic surgery*, fibrin sealant has been used for anchoring skin grafts and in conjunction with morcellised cancellous bone graft in maxillofacial reconstruction.

In *neurosurgery*, fibrin sealant has been used to augment anastomoses in extra-intracranial bypass procedures. In addition, it has been used to seal dural tears.

In *orthopaedics and traumatology*, fibrin sealant has been used in conjunction with morcellised cancellous bone graft for packing bone cavities, and in the management of epiphyseal injuries[149]. It has also been used to reduce haemorrhage in haemophiliac patients undergoing resection of pseudotumours. Peripheral nerve repairs and nerve grafting have been quite successfully carried out using fibrin sealant's adhesive qualities.

In *ear, nose and throat surgery*, fibrin sealant has been used to reduce haemorrhage in tonsillectomies and adenoid surgery in patients with bleeding disorders. It has also been used to fix middle ear bioprostheses[316] and to solve other technical problems in the surgery of the middle ear, maxillary sinuses and orbital floor.

In *Dermatology*, success has been reported in the use of fibrin sealant for cases of crural ulcers.

CHAPTER 4

Preliminary Study for the Development of a Technique for the Use of Fibrinogen Adhesive in Microvascular Anastomoses

INTRODUCTION

The characteristics of fibrinogen adhesive, presented in Chapter 3, suggest that it offers several advantages over other adhesives that have been used surgically, and its disadvantages seem to be few and relatively unimportant. In fact, put up against the criteria set out by Daniel and Terzis, fibrinogen adhesive performs quite well. For this reason, it was considered worthwhile to design and study a technique for the use of fibrinogen adhesive in making microvascular anastomoses.

Chapter 4 begins with a review of previous papers on fibrinogen adhesive microvascular anastomoses. The rest of the chapter is devoted to the description of a preliminary study in the development of a fibrinogen adhesive anastomotic technique. The first part of the preliminary study is concerned with finding a satisfactory anastomotic technique. The second part of the preliminary study is involved with the preparation of a suitable model for controlled testing of the newly developed technique.

PREVIOUS EXPERIENCE WITH ADHESIVE ANASTOMOSES

In Chapter 3 it was pointed out that a "natural adhesive" or "fibrin suture" was used as far back as the 1940's[370,420]. Its use for peripheral nerve anastomoses is quite well established[347], but, apart from an interesting study looking at the use of fibrin tubes for venous anastomoses[365], its use for vascular end-to-end anastomoses was not proposed till 1977[243]. Soon afterwards, the histological healing process of experimental anastomoses using fibrin adhesives was described[94], and it was not long before a small clinical series of arterial anastomoses was reported - eleven out of twelve anastomoses remaining patent[191].

Reference to the German publications, concerning the use of fibrinogen adhesives for microvascular anastomoses, is made in Chapter 3 (see page 74). A small number of reports are also published in the English language literature.

Some investigators have used fibrinogen adhesive to augment previous described techniques such as conventional sutured anastomoses[13,192,299], and a coupling technique[326].

Other investigators[190] attempted to use fibrinogen adhesive to seal end-to-end anastomoses approximated with only two sutures, but the thrombosis rate was very high.

Wintermantel tried using a fibrinogen adhesive to augment his experimental thermic vascular anastomoses[412].

End-to-side and side-to-side anastomoses using fibrinogen adhesive were described by Gestring, Lerner and Requena[115].

Karl, Tilgner, and Heiner[179] used a combination of end-in-end technique with fibrinogen adhesive in a small and uncontrolled study of end-in-end anastomoses. Fourteen day follow up demonstrated patency in all ten femoral arteries but in only eight out of ten femoral veins.

Brunner[43] experimented with a fibrin-glued telescoping anastomosis, made over a Fogarty embolectomy catheter.

A more recent Japanese study[363] investigated the use of 'fibrin glue' as a method for saving time during the insertion of venous interposition grafts. They demonstrated the technique to be speedy, and anastomotic failure occurred in only one out of forty-five rats. They also described a successful clinical case.

The English literature only contains brief mention of a single microvascular free tissue transfer using fibrinogen adhesive[358]. In this case, a cutaneous groin flap was transferred to the neck of a twenty-one year old man for the release of a burn contracture. A combination of suture and fibrin sealant was used at the anastomotic site.

PRELIMINARY STUDY

Introduction

A preliminary study was initially carried out to examine and develop different methods for making microvascular anastomoses. The study was aimed at two main objectives:

1. To develop a new fibrinogen adhesive anastomotic technique that offered potential advantages and was not simply a fibrinogen adhesive supplement to a conventional form of anastomosis.
2. To develop an experimental model that would be satisfactory for a controlled clinical trial of the newly designed fibrinogen adhesive anastomoses.

It was felt that the achievement of these aims was an important first step prior to carrying out a prospective experimental series comparing the new fibrinogen adhesive anastomoses to the 'gold standard' of conventionally sutured anastomoses.

Four main goals were set in order to achieve the two aims of the preliminary study:

1. Familiarisation with the standard rat and rabbit femoral artery and groin flap models.
2. Development of the new fibrinogen adhesive anastomotic technique.
3. Development of a satisfactory groin flap model.
4. Completion of a small preliminary trial of the new technique to ensure that it was feasible, that it appeared to offer potential advantages over conventional technique and that it could be reliably reproduced.

Materials and methods

The preliminary study was designed to be strictly developmental. Although an outline plan was made prior to its commencement, the study was not intended to be controlled, statistically significant, or to follow a strict protocol.

The preliminary study (Figure 6) was made up of three components: a rat study, a rabbit study and a preliminary trial.

Rat study. Initially six white laboratory rats underwent microvascular anastomoses to each femoral artery. Four types of anastomoses were made:

1. Sutured anastomoses with no fibrinogen adhesive.
2. Sutured anastomoses with fibrinogen adhesive.
3. Lauritzen sleeve anastomoses with no fibrinogen adhesive.
4. End-in-end anastomoses with fibrinogen adhesive.

A total of twelve anastomoses were made and in each case blood flow was checked intra-operatively with a patency test and a spurt test. Animals were sacrificed at the end of the experimental operations.

Rabbit study. After the rat study was completed, twelve young New Zealand White rabbits were used to further study different methods of making an end-in-end anastomosis. This was done to try to find the most efficient way of making an end-in-end anastomosis so that the best possible technique could be tested in the experimental study. Several different methods were tried out and, eventually, one method was selected as the best.

At the same time as the rabbits were being used to develop and test the adhesive anastomoses, they were also used to work out specific details of the experimental model:

PRELIMINARY STUDY

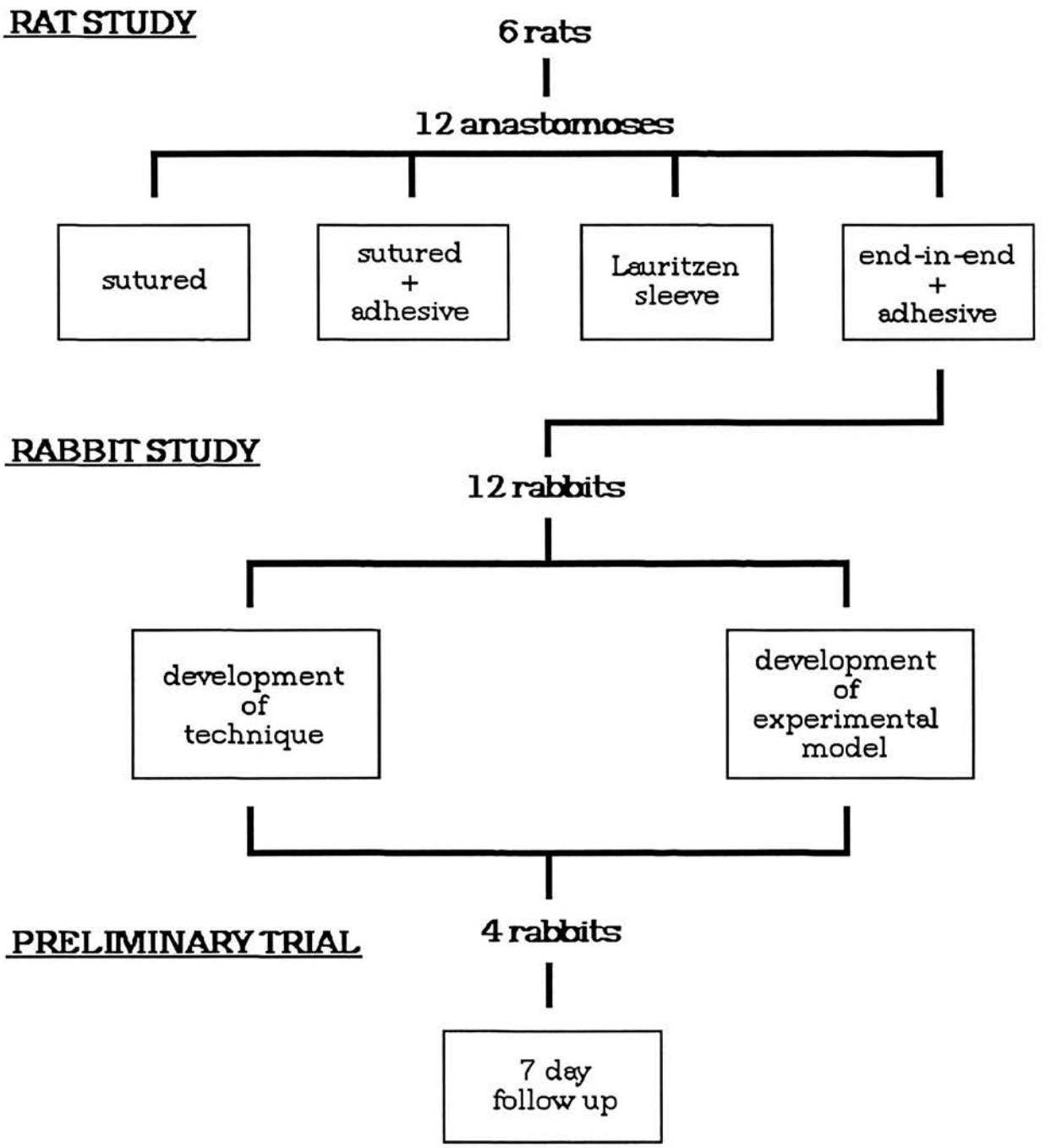


Figure 6. Diagrammatic representation of the course of the preliminary study.

1. Best size and shape for the flaps.
2. Safest anaesthetic technique.
3. Most useful follow up studies.

Early in the study, rabbits were only subjected to microvascular femoral artery anastomosis. Later, rabbits underwent groin flap transfer.

In rabbits used for microvascular anastomosis development, blood flow was checked intra-operatively with a patency test and a spurt test in a manner similar to that used in the rat sudy. The same flow tests were used in rabbits that underwent groin flap transfers, although, in addition, flap colour, capillary refill and bleeding were also assessed in these animals.

Eight rabbits were sacrificed at the end of the experimental operations. The remaining four were used for a preliminary trial.

Preliminary trial. Four animals from the rabbit study were followed for seven days in order to assess the patency of the new adhesive anastomoses after post operative recovery and one week of follow up.

Results

Development of a new adhesive technique

Rat study. Amongst the 12 anastomoses carried out in the 6 rats, the sutured anastomoses acted as a control, against which the other techniques could be compared. A standard end-to-end front wall interrupted suture technique was used. All the sutured anastomoses were patent at the end of the operative procedure.

The addition of fibrinogen adhesive did not appear to add any benefit to the sutured anastomoses.

Technical problems were encountered with the Lauritzen sleeves. The sleeve technique could not be made to work in the rat model despite Lauritzen's reported 100% success rate in rat femoral arteries. All the failures appeared to be due to excessive

intra-lumenal pressure which caused the anastomoses to leak, push the sleeve into an unsleeved position and finally result in a blow-out of the anastomosis. All femoral artery anastomoses blew-out at the time of operation.

When end-in-end anastomoses were modified and supplemented with the application of fibrinogen adhesive, all anastomoses were successfully completed.

These results confirmed my previous impression that it was difficult to reproduce Lauritzen's work with the sleeve technique. The results also suggested that a modified end-in-end technique, used in conjunction with fibrinogen adhesive, might produce a dependable new technique that could compete with a conventional sutured anastomoses.

Rabbit study. In the rabbits, the results of Lauritzen sleeve anastomoses and the modified end-in-end anastomoses with fibrinogen adhesive were similar to those in the rats. The plain sleeves did not succeed whereas the modified adhesive end-in-end anastomoses all remained patent in the short term. As more of the new adhesive anastomoses were carried out, technical details were improved and the final technique was developed.

It was decided that the new adhesive technique was satisfactory, appeared to have advantages over other techniques available, and should be tested in a full experimental study. Exact details of the technique are described and illustrated on page 109.

A small number of sutured anastomoses in rabbits were made at the beginning of the study. These were all successful, but it was noted, with interest, that the adhesive anastomoses could be completed in about half the time of the sutured anastomoses. A standard end-to-end front wall interrupted suture technique was used. It was decided to use this technique for the control in the experimental study. Exact details of the technique are described and illustrated on page 111.

Preliminary trial. In each of the four rabbits followed for seven days post-operatively, flaps remained completely viable and patency tests were positive at the time of sacrifice. This suggested that the new fibrinogen adhesive end-in-end anastomotic technique was durable and confirmed the impression that the technique was worth testing.

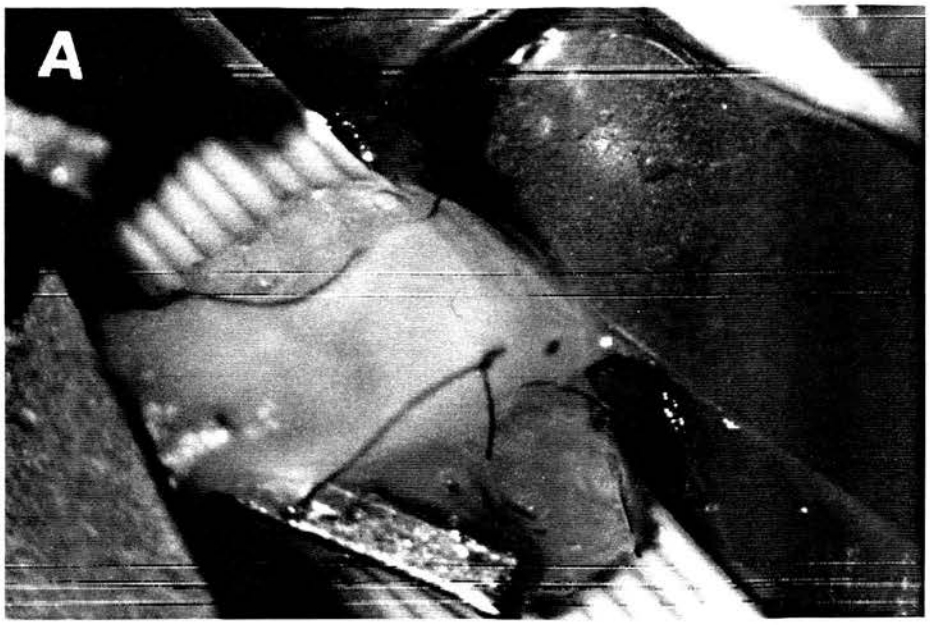
The experimental fibrinogen adhesive end-in-end anastomosis and the control sutured end-to-end anastomosis are illustrated in Figure 7.

Development of an experimental model

The experimental animal

Although the pilot study results indicated that there was no difference in patency rates, it was decided that rabbits, rather than rats, would be used in the experimental study. The main reasons for this were:

1. Rabbits were more pleasant to work with.
2. Rabbit femoral arteries were thought to be more similar to those encountered in human clinical practice.
3. Larger flaps and larger vessels were available in the rabbits on account of the animal's bigger size, and it was hoped that this would reduce the number of failures and result in better evaluation of the anastomotic technique.
4. Anaesthetic technique was thought to be associated with less peri-operative risk in the rabbits.
5. Rabbits were more reliable than rats from the point of view of long term survival.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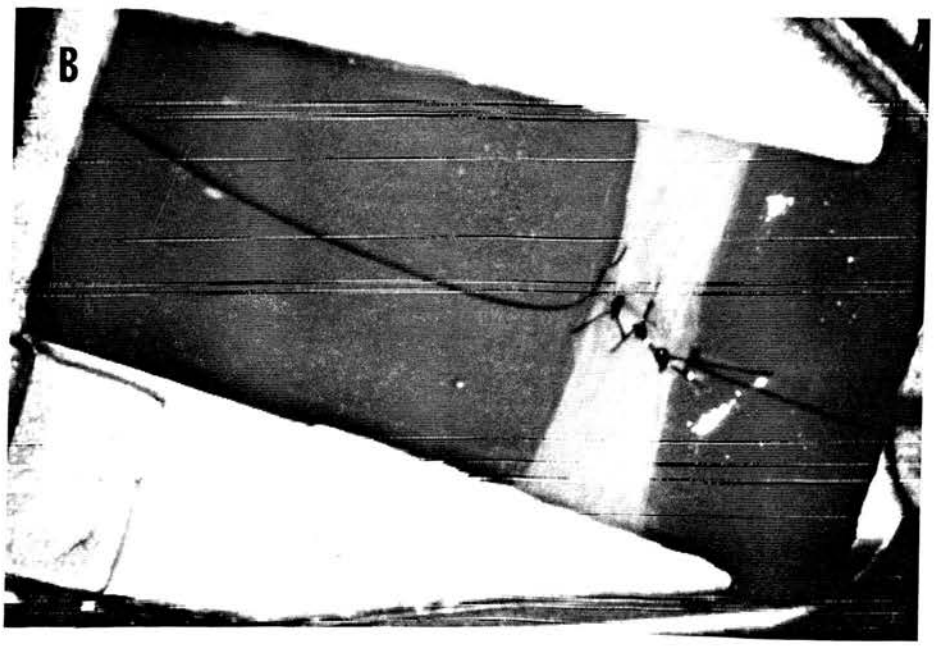
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Figure 7. The two types of microvascular anastomosis. Photographs taken through the operating microscope using x15 magnification.

A Adhesive anastomosis (Preliminary study rabbit).

B Sutured anastomosis (Preliminary study rabbit).

Young New Zealand White rabbits were used as they were available, clean, robust, healthy, and similar in size. The vascular anatomy of the region of the inguinal ligament and proximal thigh has been well described[22,40]. There is some variation in the location and pattern of arterial branches and venous tributaries. These are normally unimportant, although in a few cases they did produce technical difficulty with the anastomoses (see page 170).

Peri-operative management

Rabbit general anaesthetic technique was well known to the animal health technicians in the laboratory. They were less familiar with techniques for lengthy anaesthesia, and advice was obtained from the laboratory veterinarian. The twelve rabbits used in the preliminary study enabled the laboratory staff to perfect their anaesthetic technique. The four rabbits kept for seven post-operative days provided experience in the management of these animals post-operatively.

General anaesthetic was used with an intramuscular injection of Rompun (xylozene) 3 mg/kg and Ketamine 40 mg/kg for induction. Maintenance of anaesthesia was achieved with a mixture of 2.0 - 3.5% halothane and oxygen at 3 litres per minute delivered through a Bains facemask. The percentage of halothane varied a little both from animal to animal and also from time to time during the operative procedure.

Intravenous fluids were used intra-operatively for some of the initial operations but were soon discontinued as the operations were not as lengthy as first expected, and operative fluid loss was minimal.

The operating room arrangement is illustrated in Figure 8.

Post-operatively the rabbits were recovered overnight in warm padded intensive care cages. The following day they were returned to their regular holding cages. It was necessary to tape light bandages circumferentially around the animals' abdomen in

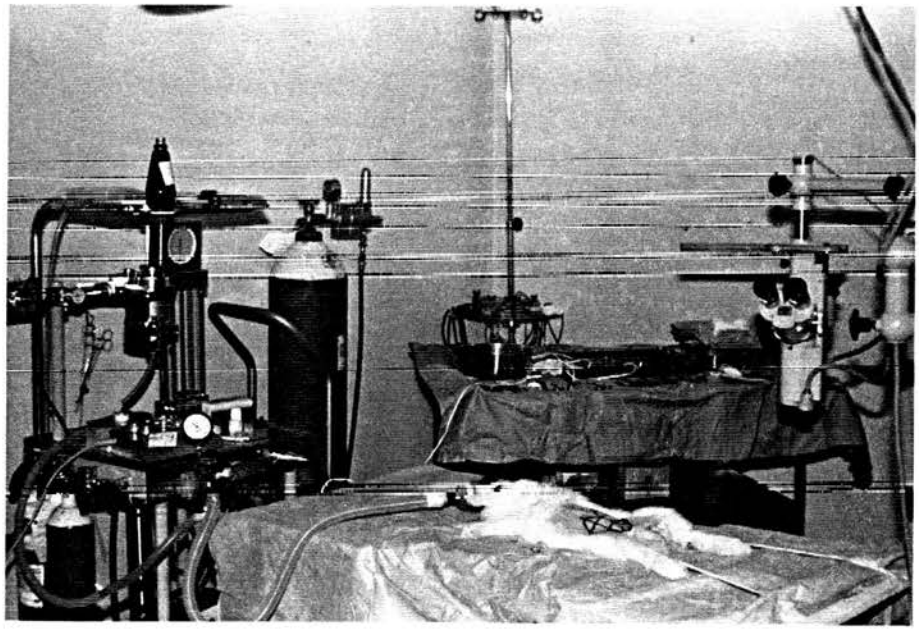


Figure 8. Photograph illustrating the operating room arrangement. The rabbit (R24) is supine on the operating table and is receiving anaesthetic through a Bains facemask. The operating microscope is to the right of the picture and the surgical instruments are on a side table.

order to prevent the flaps from dragging along cage floors. Dressings were inspected daily and changed as frequently as necessary till the wounds were completely healed.

The rabbits had no problem returning to their normal diets in the immediate post-operative period.

Post-operative infections were not seen and, thus, it was felt that the administration of antibiotics would not be necessary for wound healing.

Similarly, the animals did not appear to be in discomfort post-operatively and so neither analgesics nor sedation were used.

Development of the experimental flap

Having used the femoral arteries for the development of the anastomotic technique, it was a logical progression to consider the use of the groin flap for the experimental model.

The anatomy of the rabbit groin is not unlike that of the human. The femoral artery originates as a continuation of the external iliac artery beneath the inguinal ligament and passes through the anterior and medial aspect of the thigh on its route to the popliteal fossa. In the femoral triangle it gives off a number of branches including the epigastric artery, which supplies the skin of the abdominal wall and supports the groin flap. Venous drainage follows the arterial supply.

Rounded flaps were favoured over rectangular flaps in order to avoid pointed corners at the margins. In the size of animals used, it was found that elliptical flaps measuring 5cm x 7cm seemed to be the best size to enable bilateral and symmetrical flaps to be raised. These flaps were adequately sized for a free tissue transfer model.

The main problem with this model was that the venous anastomoses could not be considered representative of human vascular repairs due to their very thin walls. There was concern that these flimsy vessels might skew the results of the

experimental study by lowering the patency rates, reflecting a problem with the model rather than the anastomotic techniques. To avoid this source of error, and to make the results easier to interpret, it was decided to use the groin flap model with only an arterial anastomosis, leaving the veins intact. All other structures were divided and thus the flaps could be considered as being either modified island flaps or modified free flaps, their form lying part-way between the two types of flap.

Follow up evaluation

During the preliminary study, thought was also given to the best method for evaluation of the anastomoses post-operatively.

There was no doubt that flaps were useful as they provided a method of day-to-day monitoring. The shaved skin of the white rabbits was quite easy to subjectively evaluate for blood flow as it clearly became pink in colour after the arterial clamps were removed. Most of the flaps contained one or two nipples and these were found to be particularly useful as their colour was easy to assess, and they also visibly increased their turgor with the re-establishment of blood flow.

At sacrifice it was initially planned to assess anastomotic patency using a standard patency test[4,11,150] (bearing in mind its possible inaccuracies[267]), arteriography (with either renografin or barium) and to make and study intra-lumenal latex casts[178,383]. Concern was raised, however, that this might lead to difficulties with the processing and interpretation of histological studies of the vessel wall. For this reason, it was decided to substitute the arteriography and intra-lumenal latex cast techniques with scanning electron microscopy of the intra-lumenal surface of the anastomoses. Several of the anastomoses in the preliminary study were removed and used to establish satisfactory processing techniques in the histology and scanning electron microscopy laboratories. Exact technical details are described on page 128.

THE EXPERIMENTAL TECHNIQUE WITH FIBRINOGEN ADHESIVE

The experimental fibrinogen adhesive anastomotic technique is diagrammatically illustrated in Figure 9.

Using the magnification of a Zeiss operating microscope, the femoral vessel was clamped and divided in a microvascular double approximating clamp. The cut ends of the femoral vessel were then approximated without tension and cleaned of peri-adventitial tissue. Care was taken to ensure that there was adequate vessel length for the anastomosis and that there were no branches near the cut vessel ends in a location that would interfere with the smooth formation of an end-in-end anastomosis.

Two stay sutures of 10-0 dermalon (Davis and Geck) with 100 micron needles were inserted 180^{0} apart, at 3 o'clock and 9 o'clock, in such a manner as to produce an end-in-end anastomosis with one vessel passing into the other for a distance of one to two diameters. The proximal end of the artery (up-stream vessel) was inserted into the distal end (down-stream vessel). The stay sutures were placed full thickness through the outer vessel wall, but only partially (into the media but not the adventitia) into the inner vessel wall. The stay sutures were tightened and knotted. To prevent loss of overlap in the back wall, due to retraction of the outer (down-stream) vessel wall, a retracting suture was temporarily placed full thickness through the free edge of the back wall centrally (at 6 o'clock). The outer surface of the anastomosis was then sealed with a film of adhesive (made up from a Tisseel Kit 0.5, using Thrombin 500), applied using the Duploject system. The completed anastomosis was left for three minutes, to allow the adhesive to fully set, before the retracting suture was removed and the microvascular clamps were released. The final anastomosis is illustrated in Figure 7.

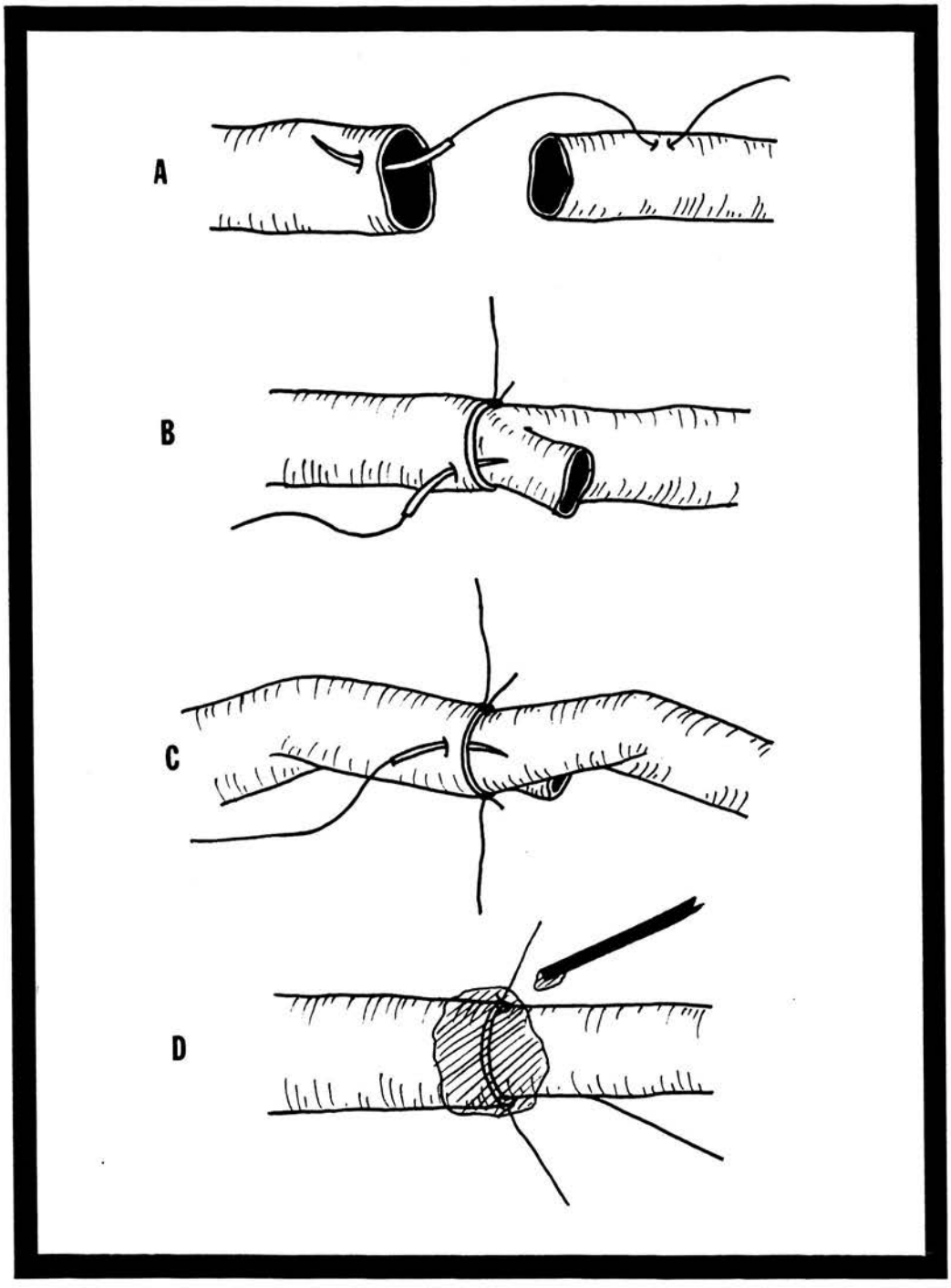

A
B
C
D

Figure 9. Diagrammatic representation of the technique used to make the adhesive end-in-end microvascular anastomosis. In each diagram, the upstream (inner) vessel is on the right and the downstream (outer) vessel is on the left.

A Insertion of the first stay suture at the 3 o'clock position. Note that the suture passed partially through the inner vessel wall and completely through the outer vessel wall.

B Insertion of the second stay suture at the 9 o'clock position.

C The temporary retracting suture was placed at the 6 o'clock position, but only into the outer vessel.

D After the inner vessel was placed into the outer vessel, the outer surface of the anastomosis was sealed with a film of fibrinogen adhesive applied using the Duploject system.

THE CONTROL TECHNIQUE WITH SUTURES

The control sutured end-to-end anastomotic technique is diagrammatically illustrated in Figure 10.

In a manner similar to the experimental side, using the magnification of a Zeiss operating microscope, the femoral vessel was clamped and divided in a microvascular double approximating clamp. The cut ends of the femoral vessel were then approximated without tension and cleaned of peri-adventitial tissue. Care was taken to ensure that there was adequate vessel length for the anastomosis and that there were no branches near the cut vessel ends, in a location that would interfere the suture anastomosis.

Interrupted 10-0 dermalon sutures on a 100 micron needle were inserted and tied using a standard front wall technique. Two sutures were initially placed 180^{0} apart at 3 o'clock and 9 o'clock. Next a central suture was inserted at 12 o'clock. The gaps between were then sutured, usually with one suture per gap. The double approximator clamp was then rotated to present the back wall. A central suture was placed at the 6 o'clock position and, similar to the front wall, the gaps between the 6 o'clock and the lateral 3 o'clock and 9 o'clock sutures were sutured closed. Eight to ten sutures were usually needed for each femoral vessel. Finally, flow was re-established with release of the microvascular clamps in a manner identical to the experimental side. The final anastomosis is illustrated in Figure 7.

THE RABBIT GROIN FLAP EXPERIMENTAL MODEL

Rabbits were placed supine on the operating table with their lower limbs symmetrically abducted and their abdomens and thighs shaved.

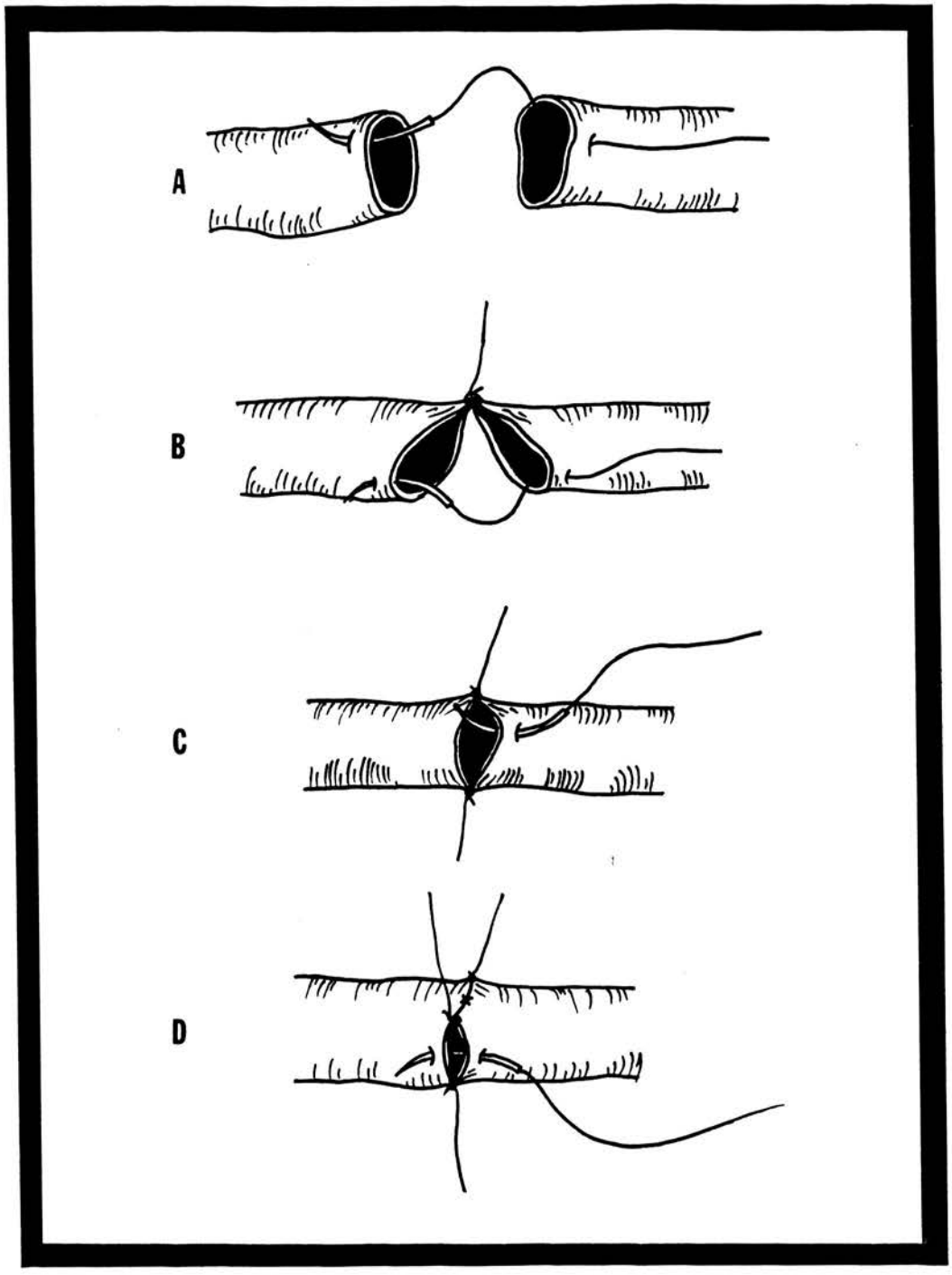

A
B
C
D

Figure 10. Diagrammatic representation of the technique used to make the sutured end-to-end microvascular anastomosis.

A Insertion of the first stay suture at the 3 o'clock position.

B Insertion of the second stay suture at the 9 o'clock position.

C Insertion of the central suture at the 12 o'clock position.

D Sutures were inserted to close the gaps between the 3 o'clock and 12 o'clock and the 12 o'clock and 6 o'clock sutures. After the front wall was completed, the microvascular clamp was rotated and steps C anc D were repeated for the back wall.

A bilateral groin flap model was designed with one side experimental and the other side control. The model is illustrated in Figure 11.

Using an elliptical template, 5cm x 7cm flaps were drawn out on the abdomen, symmetrically and with reference to the mid line and the mid points of the inguinal ligaments as illustrated in Figure 12. Flaps were marked into six segments in order to facilitate wound closure, and to aid in the identification of potential zones of failure. One segment was blacked in to aid orientation during wound closure so as to prevent malrotation and torsion of the vascular pedicle. Surgical antisepsis was achieved with Betadine solution and then all but the operative field was covered with sterile drapes.

Initially the lower part of the flap was raised and the femoral vessels located distally. These were then mobilized from the inguinal ligament proximally to a point beyond the origin of the epigastric vessels distally. Care was taken to avoid any excess tissue handling in the region of the epigastric pedicle. The femoral vessels were ligated and divided distal to their epigastric branches. The femoral vein was then separated from the femoral artery for 1.0 to 1.5 centimetres between the inguinal ligament and the inferior epigastric vessels. Branches were cauterised with bipolar cautery.

Next the remainder of the flap was raised from the lower abdominal wall, starting proximally and taking care to remain deep to the inferior epigastric vascular pedicle. The groin flap was now an island flap based on the femoral vessels and epigastic pedicle. The flap is diagrammatically illustrated in Figure 13.

A double approximating microvascular clamp was next applied proximal to the inferior epigastric pedicle, and the clamped femoral artery was divided. The microvascular anastomosis was then made, the clamp released, and flow re-established. Lidocaine 1% (1.0 to 2.0 millilitres) was applied to the

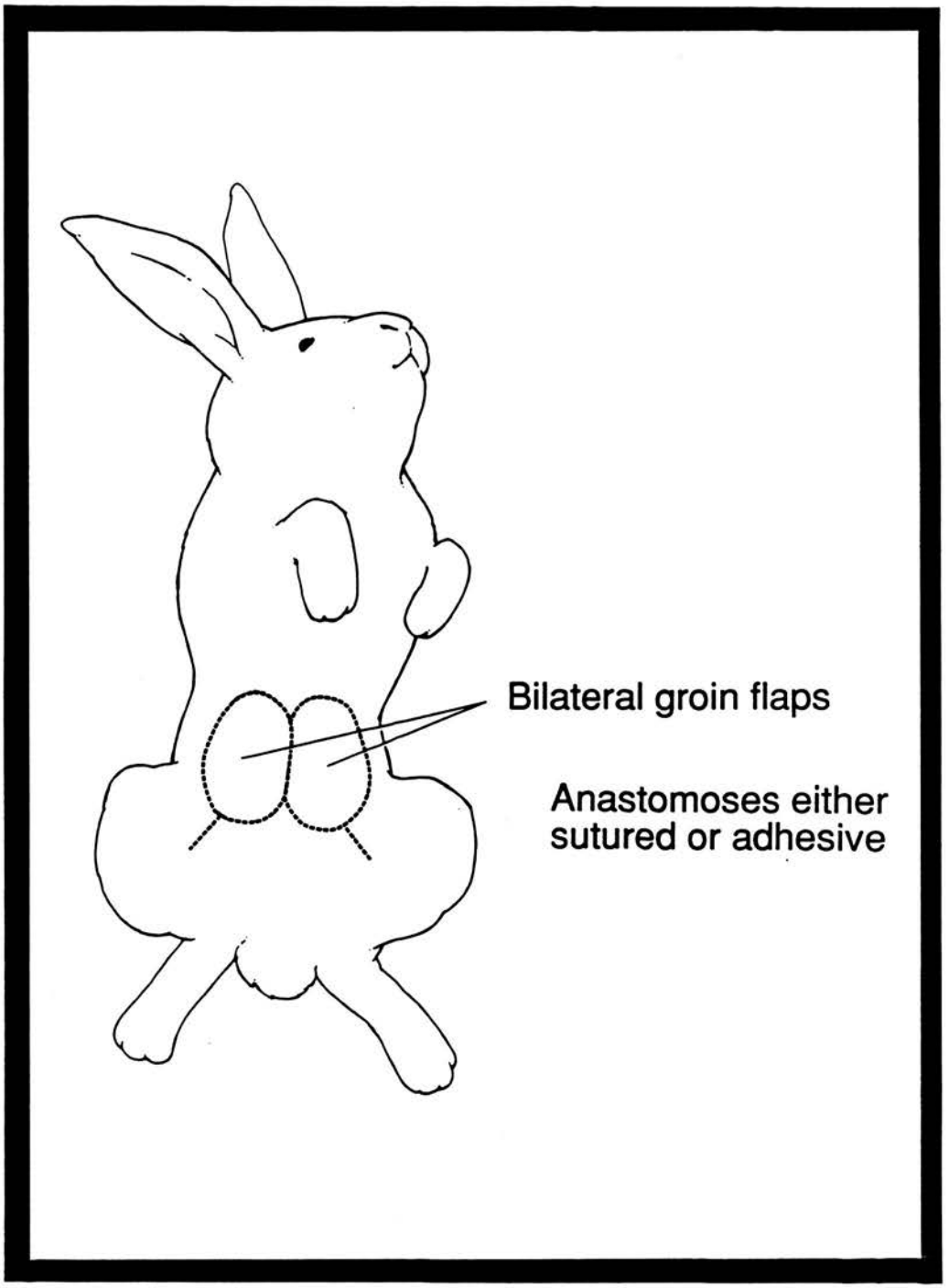

Bilateral groin flaps
Anastomoses either sutured or adhesive

Figure 11. Diagrammatic representation of the experimental animal. The groin flap on the experimental side was revascularised using a fibrinogen adhesive anastomosis, whilst the groin flap on the control side was revascularised using a standard suture technique.

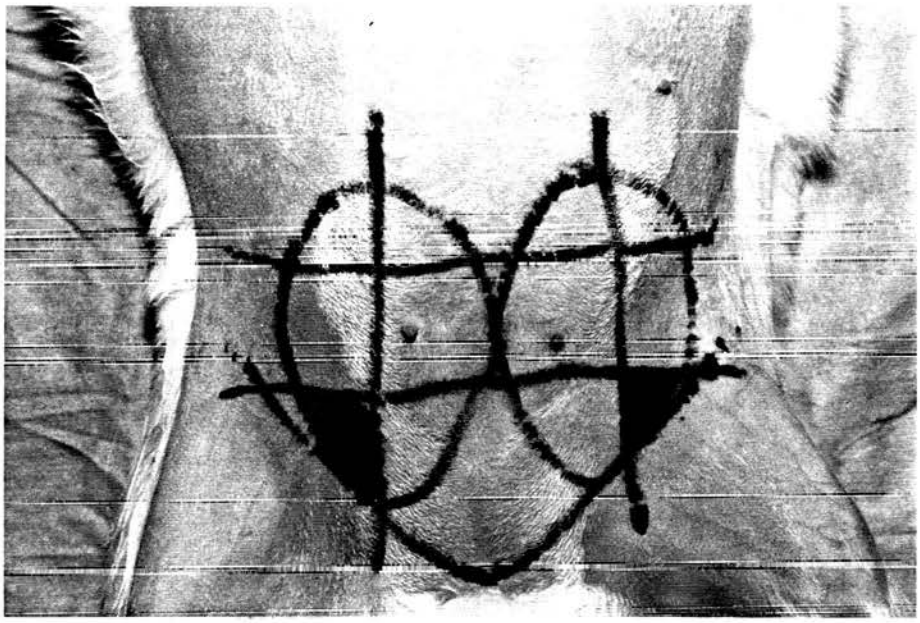


Figure 12. The experimental and control flaps. Prior to starting the operation, a plastic template was used to draw the experimental and control flaps on the rabbit's abdomen, using the pubic symphysis and anterior superior iliac spines for bony landmarks. The grid was used as an aid in wound closure and for harvesting skin biopsies. The blacked in segment helped to prevent flap rotation at the time of wound closure (R42).

Note the nipples in the flaps. These were useful in assessing capillary refill after the microvascular clamps were released.

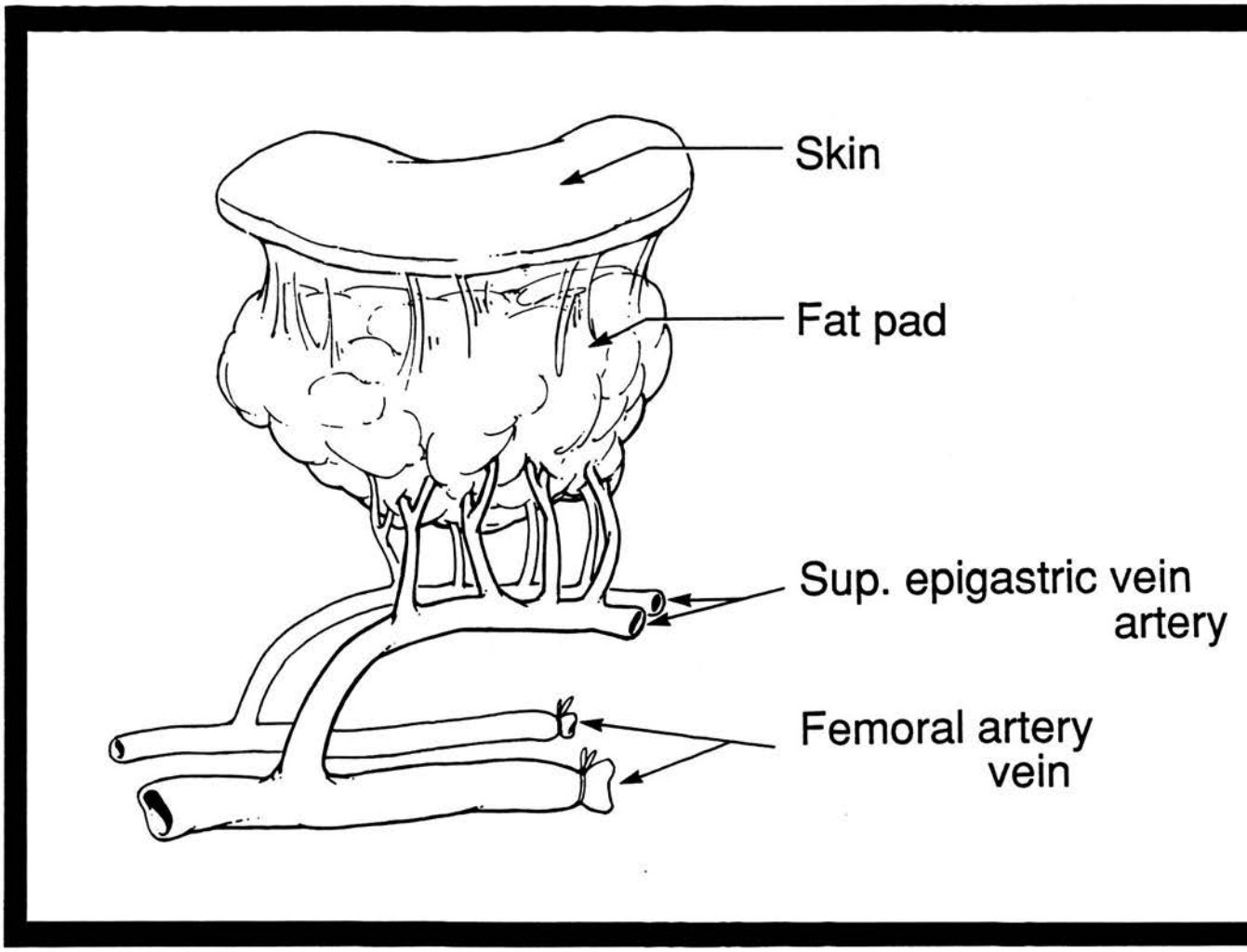

Skin
Fat pad
Sup. epigastric vein
artery
Femoral artery
vein

Figure 13. Diagrammatic representation of the rabbit groin flap experimental model. The flap was supported by the axially orientated epigastic vessels and was revascularised with a femoral artery anastomosis.

pedicle. After a few minutes a patency test was carried out to ensure that there was flow across the anastomosis. The flap was then sutured into its original location, using 3-0 silk sutures. The previously marked segmental grid was useful for achieving correct flap orientation.

CHAPTER 5

Experimental Study Comparing Fibrinogen Adhesive Technique with Conventional Sutured Anastomosis Technique

INTRODUCTION

Although there have been a small number of previous publications concerned with the use of fibrinogen adhesive for making microvascular anastomoses, the experiments and clinical cases described have been uncontrolled, and the numbers have not been large enough to consider that a satisfactory trial has yet been undertaken.

Chapter 5 describes a large and controlled experimental study aimed at comparing the new fibrinogen adhesive technique, described in Chapter 4, with a conventional suture technique, the gold standard of microvascular anastomosis. The chapter is set out in the conventional manner associated with the publication of scientific papers: an introduction (in this case listing the basic concepts behind the experimental design), materials and methods, results, discussion and conclusions.

BASIC CONCEPTS

A number of basic concepts were considered to be important to the experimental study, and these were incorporated into its design:

1. Once started, the series had to be continued as a consecutive and prospective study.
2. The experiment had to be tightly controlled. In the chosen design each animal was similar in strain, size and sex, and each acted as its own control.
3. The number of anastomoses studied had to be sufficiently large to provide statistically significant results.

4. The number of parameters examined and measured for each anastomoses had to be as large and varied as possible in order to fully evaluate the outcome for each anastomosis.
5. Potential problems had to be anticipated and precautions taken to minimise bias in the results.

MATERIALS AND METHODS

General plan

The overall plan of the experiment is illustrated in Figure 14.

Young adult female New Zealand White rabbits, weighing approximately 2.5 - 3.0 kg, were used for the experimental model.

Each animal underwent bilateral microvascular flap transfers, on the experimental side with a fibrinogen adhesive end-in-end anastomosis and on the control side with a sutured end-to-end anastomosis. This produced a controlled, consecutive, prospective series of one hundred flaps in fifty rabbits. Individual animals were easily identified by sequential numbers tattooed on the inner aspect of their ears.

Animals were divided into three follow up groups according to the length of follow-up:

1 day group ..... 10 rabbits
7 day group ..... 10 rabbits
21 day group ..... 30 rabbits

Specific identification for the rabbits in each group is documented in Table 2.

At the end of the follow up period, the rabbits were given an anaesthetic and, after the flaps had been observed clinically and

EXPERIMENTAL STUDY

OPERATION

50 rabbits

100 flaps/anastomoses

1 day follow up	7 day follow up	21 day follow up
10 sutured 10 adhesive	10 sutured 10 adhesive	30 sutured 30 adhesive

SACRIFICE

100 flaps

1. survival
2. capillary refill
3. bleeding
4. skin biopsy

100 anastomoses

1. patency test
2. gross pathology
3. laboratory studies

LABORATORY

skin biopsies

1. histology

anastomoses

1. histology
2. SEM

Figure 14. Diagrammatic representation of the experimental study illustrating details of the experimental plan, the tests carried out at the time of sacrifice and the type of laboratory investigations used to examine the flaps and anastomoses.

TABLE 2. Specific rabbit identification numbers for each of the three experimental groups.

1 day follow up

R4	R5	R6	R7	R16
R17	R30	R31	R32	R33

7 day follow up

R8	R9	R10	R11	R12
R13	R14	R15	R28	R29

21 day follow up

R18	R19	R20	R21	R22
R23	R24	R25	R26	R27
R34	R35	R36	R37	R38
R39	R40	R41	R42	R43
R44	R45	R46	R47	R48
R49	R50	R51	R52	R53

Substitutions for intra-operative failure

R14 with 114

R39 with 139 and 139 with 239

R46 with 146

skin biopsies taken, the anastomoses were re-explored and patency tests repeated. This done, the rabbits were sacrificed and the anastomoses were harvested and prepared for histological examination and scanning electron microscopy studies.

A number of potential sources of error were anticipated prior to commencement of the study. These were handled as follows:

1. In order to eliminate potential errors arising from variation in surgical expertise, all operations were carried out by a single, experienced microvascular surgeon (myself).

2. In order to eliminate any bias of side or order, a convention was established for deciding which flap (left or right; first or second) would have a sutured anastomosis and which would have a fibrinogen adhesive anastomosis. The left flap was always done first and the type of anastomosis was determined by referring to a computer generated table of random numbers (Table 3). An even number indicated that the left (first) flap would have a fibrinogen adhesive anastomosis, whereas an odd number indicated that the left (first) flap would have a sutured anastomosis. The specific matching of experimental animals to random numbers is shown in Table 4.

3. In order to minimise my own 'learning curve' error, the consecutive series was not started till I felt satisfied that my technical ability to carry out the new fibrinogen adhesive anastomosis equalled my ability to complete a conventional suture anastomosis (hence the false start and the matching of the first rabbit - R5 - with the random number 1470).

Anaesthetic

The anaesthetic technique used was identical to that described in Chapter 4 for the rabbit groin flap experimental model (see page 105).

TABLE 3. Table of random numbers used to determine whether the arterial anastomosis for the left (first) flap would be made using suture technique or the fibrinogen adhesive technique.

14	99	461	513
814	842	850	922
934	1041	1176	1370
1470	1560	1608	1769
1789	1848	1904	1906
1924	1992	2040	2051
2136	2257	2388	2410
2724	2878	2897	3246
3279	3333	3484	3494
3547	3570	3635	3736
3901	3926	3928	4269
4322	4347	4402	4500
4545	4752	4934	5027
5076	5118	5170	5216
5346	5508	5537	5559
5670	5894	5914	6050
6094	6321	6462	6474
6479	6605	6675	6806
7059	7072	7178	7223
7310	7416	7424	7452
7455	7559	7718	7995
8138	8139	8191	8320
8404	8431	8478	8723
8869	8932	8940	8961
9016	9383	9697	9811

TABLE 4. The manner in which the consecutive and prospective series of experimental rabbits were matched to the computer generated table of random numbers.

14	-	99	**R27**	461	**R52**
814	-	842	**R28**	850	**R53**
934	-	1041	**R29**	1176	**R54**
1470	**R5**	1560	**R30**		
1789	**R6**	1848	**R31**		
1924	**R7**	1992	**R32**		
2136	**R8**	2257	**R33**		
2724	**R9**	2878	**R34**		
3279	**R10**	3333	**R35**		
3547	**R11**	3570	**R36**		
3901	**R12**	3926	**R37**		
4322	**R13**	4347	**R38**		
4545	**R14**	4752	**R39**		
5076	**R15**	5118	**R40**		
5346	**R16**	5508	**R41**		
5670	**R17**	5894	**R42**		
6094	**R18**	6321	**R43**		
6479	**R19**	6605	**R44**		
7059	**R20**	7072	**R45**		
7310	**R21**	7416	**R46**		
7455	**R22**	7559	**R47**		
8138	**R23**	8139	**R48**		
8404	**R24**	8431	**R49**		
8869	**R25**	8932	**R50**		
9016	**R26**	9383	**R51**		

Operative technique

The operative technique used was identical to that described in Chapter 4 for the rabbit groin flap experimental model (see page 111), the experimental fibrinogen adhesive anastomoses (see page 109) and the control sutured anastomoses (see page 111). Pre-operative markings, drawn around the plastic template, ensured that flap size and location was always the same, and segmental grids were drawn on as a technical aid (Figure 12).

Both experimental and control sides were operated on immediately consecutively as a single operative procedure. A clock was started at the beginning of the operation, and the times of key events, such as skin incision, start and finish of microvascular anastomoses, and skin closure were recorded. Specific technical details concerning each procedure were also noted. The collection of this information, together with the maintenance of post-operative progress notes, ensured that complete and individual records were available for each animal, operation, flap, and anastomosis.

Intra-operative flap viability was checked by assessing the cutaneous capillary refill and the degree of bleeding, after release of the microvascular clamps. In addition, a venous outflow patency test was used as a test of blood flow.

Sutured anastomoses that were thought to be unsatisfactory at the time of operation were resected and replaced. It was not possible to do this with fibrinogen adhesive anastomoses because of constraints which were thought to be specific to the model and not relevant to human clinical practice (see page 172). For this reason, on the small number of occasions when fibrinogen adhesive anastomoses were thought to be unsatisfactory at the time of operation, operations were abandoned and the animal was replaced. In this manner, all animals admitted to the study were good working models containing functioning experimental and control anastomoses. This protocol ensured that the models started out on

an equal basis and was felt to be acceptable because it represented the clinical principle that unsatisfactory anastomoses must be either revised or replaced. In order to ensure that this protocol did not bias results in favour of the adhesive anastomoses, a record of intra-operative technical failures was kept, and is reported in the results section (see page 134).

Post-operative management

The post-operative management was identical to that described in Chapter 4 for the rabbit groin flap experimental model (see page 105).

Intravenous fluid was not given post-operatively, and rabbits returned to their normal diet immediately after recovering from their anaesthetic.

The flaps were inspected daily and dressings were changed according to clinical need until the wounds were healed, at which time dressings were discontinued. Close attention was paid to wound healing and appropriate nursing and/or surgical measures were taken in the small number of cases where this proved to be necessary.

Antibiotics were not used routinely but in cases where there were clinical problems with wound healing (partial or complete flap necrosis), tribrissen 24% was administered subcutaneously in a single dose of 30 mg/kg/day till the problem was resolved.

Analysis of results

Technical data, gathered at the time of each operation, included the animal's weight, external diameter of femoral arteries, total operating time for each flap, and time taken to complete each anastomosis (experimental and control). Notes were made of technical differences (both positive and negative) between the two techniques. Intra-operative complications were recorded. Intra-operative flow tests were carried out both at the distal side of the

arterial anastomosis and through the draining femoral vein. In addition, at the end of each operation, flap bleeding was noted, and flap colour and capillary refill recorded.

Daily clinical observations of flap viability and fur growth were recorded in the clinical chart of each individual animal.

On the day of sacrifice (either 1 day, 7 days or 21 days) each animal was again given a general anaesthetic using the same technique used for the original operative procedures. Flaps where photographed (Figure 15), and objective studies of flap survival and anastomotic patency were then undertaken.

Skin biopsies were taken from both the distal (cranio lateral) and proximal (caudo medial) end of each flap. Biopsies were cut full thickness through skin and subcutaneous tissues and measured approximately 2.0 mm x 2.0 mm. They were immediately placed in a solution of gluteraldehyde 5% in 0.2M s-collidine buffer. Skin biopsy sites were checked for bleeding, and this was photographed (Figure 16).

Experimental and control flaps were then dissected from the abdominal wall and the epigastric vascular pedicles carefully removed from surrounding scar tissue. With the flaps now isolated as islands on their intact vascular pedicles (Figure 16), circulation was again checked by looking at the bleeding from skin biopsy sites and capillary refill. A venous outflow patency test was next carried out and this was followed by a spurt test produced by transecting the epigastric arteries distal to the anastomoses. Animals were then sacrificed with an overdose of sodium pentothal.

The anastomotic sites were rapidly fixed with a solution of gluteraldehyde 5% in 0.2M s-collidine buffer, and removed en bloc. Using the operating microscope, the artery was dissected from the adjacent vein and surrounding scar tissue. Arteries were then divided longitudinally, from proximal to distal, and split into two halves. A small suture was placed in the proximal end of each to mark the direction of flow. The lumenal surface of each half artery

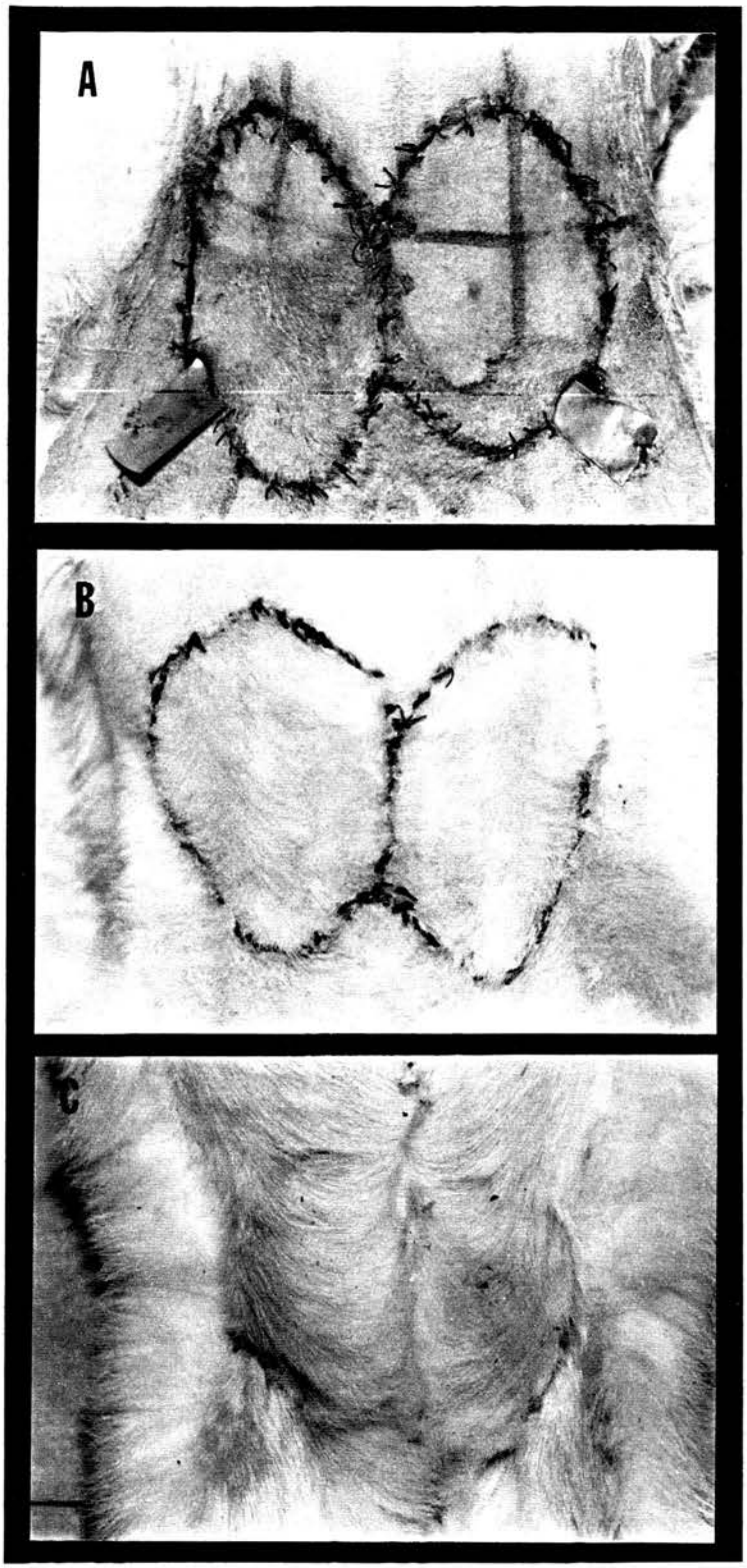

A
B
C

Figure 15. Photographs demonstrating the appearance of flaps at the time of sacrifice.

A Flaps at 1 day (R30).

B Flaps at 7 days (R29).

C Flaps at 21 days (R24).

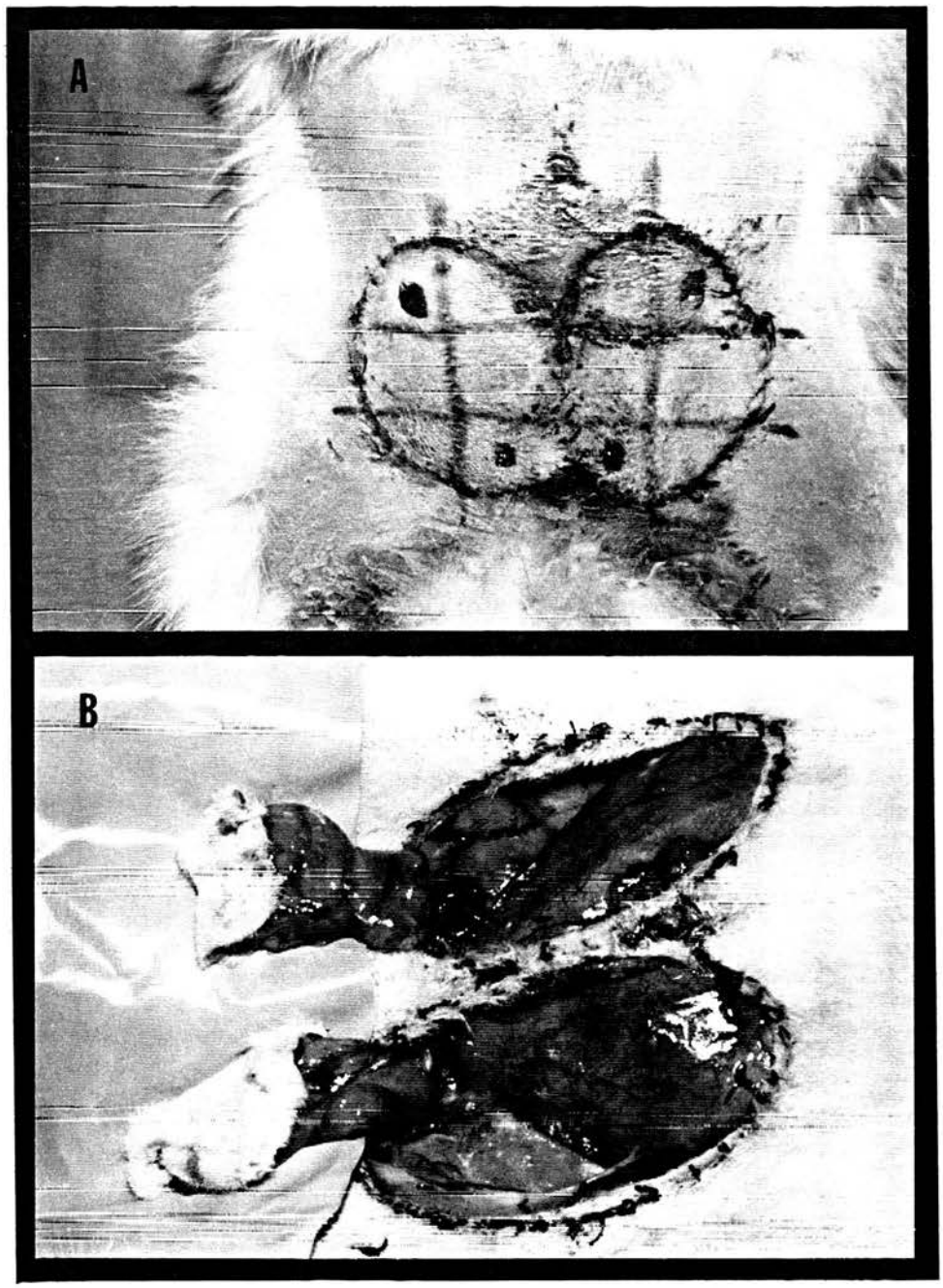

A
B

Figure 16. Photographs of flaps at the time of sacrifice.

A Shaved flaps at 7 days demonstrating skin biopsy sites (R8).

B The flaps have been isolated as islands on their vascular pedicles. The venous outflow patency test and spurt test were about to be carried out (R28).

was gently flushed with normal saline solution to remove any freshly formed blood clot, but care was taken not to wash away any mural thrombus formation. Specimens were then placed into a solution of 5% glutaraldehyde in 0.2M s-collidine buffer.

All specimens (skin biopsies and arterial sections) were stored at 4°C until processed in the histology laboratory. Processing consisted of:

1. Samples were washed with 0.2 M s-collidine buffer pH 7.3 (using 3-4 changes of Buffer over a minimum of three days).
2. The material was then postfixed with 1% osmium tetroxide in freshly prepared sodium bicarbonate buffer for one hour at room temperature.
3. The samples were rinsed quickly with distilled water and then,
4. dehydrated in a graded series of ethanol 50%, 70%, 95%, 100%, 100% for one hour each.
5. This was followed by substitution to amyl acetate, placing the samples first in a mixture of one part amyl acetate to three parts ethenol for 30 minutes, then in a mixture of one part amyl acetate to one part ethenol for 30 minutes, then into pure amyl acetate.

Arterial sections, to be processed for scanning electron microscopy, were stored at room temperature in amyl acetate until they could be critical point dried, mounted on studs and gold coated for viewing in a Philips SEM 505 scanning electron microscope.

The other half of each fixed artery sample was imbedded in methyl methacrylate and then cut to 1.0 to 1.5 microns. These

were then stained with haemotoxylin and eosin and toluidine blue for light microscopic examination.

Skin biopsy specimens were also imbedded in methyl methacrylate, cut to 1.0 to 1.5 microns, and stained with haemotoxylin and eosin and toluidine blue for light microscopic examination.

Details of composition of solutions used in the histology laboratory are listed in Table 5.

RESULTS

General results

The experiment used fifty-four young adult female New Zealand White rabbits weighing between 1.25 and 3.75 kg (mean weight 2.06 kg). The four extra rabbits were used to replace cases where fibrinogen adhesive anastomoses failed intra-operatively (R14 replaced by 114; R46 replaced by 146; R39 replaced by 139, which was again replaced by 239).

Four rabbits (all in the 21 day group) did not complete their follow up period due to non-microsurgical post-operative complications. Three had severe wound infections (R18; R45; 239), and one suffered an unexpected cage death (R49). There were no other post operative problems.

Femoral artery diameter

There was some variation in femoral artery diameter amongst the different animals. On the sutured side, femoral artery diameter varied from 1.0 to 1.8 mm (mean diameter 1.2 mm), whilst on the adhesive side, femoral artery diameter varied from 1.0 to 1.8 mm (mean diameter 1.2 mm). Statistically, these differences in femoral artery diameter were not significant (paired t value 0.544, DF 48, NS).

TABLE 5. Details of the buffer and fixative solutions used in the histology laboratory.

Buffers:

bicarbonate buffer

- 2.5% $NaHCO_3$ adjust pH to 7.2 - 7.4 just before using with 1N HCl.

s-collidine buffer (0.2M)

- 2.67 ml pure s-collidine (2-4-6 trimethyl pyridine).
- 50 ml distilled water.
- approximately 9 ml 1N HCl.

Adjust pH to 7.2 - 7.4 with1N HCl and dilute to 100 ml with distilled water (this makes a stock buffer that is stable for storage under refrigeration).

Fixatives:

5% gluteraldehyde in 0.2M s-collidine buffer

- 20 ml of 25% solution of gluteraldehyde, bring volume up to 100ml with stock 0.2M s-collidine buffer.

1% osmium tetroxide in sodium bicarbonate buffer

- one part stock 4% osmium tetroxide in water (1 gm OsO_4 in 25 ml water).
- one part distilled water.
- two parts 2.5% sodium bicarbonate buffer.

Intra-operative technical failures

Four of the sutured anastomoses were not considered to be acceptable at the time of operation. These were resected and replaced. Three of these (R43; R50; R51) went on to a successful outcome, whilst the fourth (R49) appeared to be successful, although this could not be confirmed completely as the animal died, unexpectedly, in the cage before the completion of follow up.

Three of the fibrinogen adhesive anastomoses were not considered to be acceptable at the time of operation. These models were replaced with new animals. Two of these (114; 146) went on to a successful outcome, whilst the third (239) became infected, and was sacrificed before the completion of follow up.

Anastomotic speed

The time taken to complete a sutured anastomosis varied from 15 to 37 mins (mean time 21.1 mins), whereas the time taken to complete an adhesive anastomosis varied from 11 to 22 mins (mean time 14.7 mins).

Statistically, the time taken to complete fibrinogen adhesive anastomoses was significantly less than for sutured anastomoses (paired t value 8.864, DF 46, $p<0.0005$).

Total operating time for flaps with a sutured anastomosis varied from 36 to 60 mins (mean 46.9 mins), whereas total operating time for flaps with a fibrinogen adhesive anastomosis varied from 32 to 51 mins (mean 41.4 mins).

Statistically, total operating time for flaps with fibrinogen adhesive anastomoses was significantly less than for flaps with sutured anastomoses (paired t value 4.979, DF 46, $p<0.0005$).

Flap survival

Individual results of the 1 day follow up group are listed in Table 6. On the sutured anastomoses side, 10/10 flaps survived

TABLE 6. Individual results of the 1 day follow up group.

No problem either side:

R4	R6	R7	R16
R30	R31	R32	R33

Unilateral problems - suture anastomosis:

R17 partially thrombosed anastomosis - flap survival

Unilateral problems - fibrinogen adhesive anastomosis:

R5 completely thrombosed anastomosis - partial flap necrosis (primarily venous flow failure)

TABLE 7. Individual results of the 7 day follow up group.

No problem either side:

R8	R9	R10	R11
R12	R13	R15	R28
R29			

Unilateral problems - fibrinogen adhesive anastomosis:

R14 intra-operative failure - successful revision with 114

TABLE 8. Individual results of the 21 day follow up group.
(Underlining indicates failure to complete follow-up)

No problem either side:

R19	R20	R22	R23
R24	R26	R27	R35
R36	R37	R38	R40
R42	R44	R48	R52
R53			

Bilateral problems:

R18 infected

R45 infected

Unilateral problems - suture anastomosis:

R43 intra-operative failure - successful revision

R50 intra-operative failure - successful revision

R51 intra-operative failure - successful revision

R49 intra-operative failure - successful revision - cage death

R47 partially thrombosed anastomosis - flap survival

Unilateral problems - fibrinogen adhesive anastomosis:

R46 intra-operative failure - successful revision with 146

R39 intra-operative failure - revision with 239 - infected

R41 anastomosis partially unsleeved - flap survival

R25 partially thrombosed anastomosis - flap survival

R21 completely thrombosed anastomosis - partial flap necrosis

R34 completely thrombosed anastomosis - complete flap necrosis

completely, whereas on the fibrinogen adhesive anastomoses side 1/10 flaps (R5) underwent partial necrosis.

Individual results of the 7 day follow up group are listed in Table 7. On the sutured anastomoses side, 10/10 flaps survived completely, and 10/10 flaps on the fibrinogen adhesive side also survived completely.

Individual results of the 21 day follow up group are listed in Table 8. Amongst the surviving animals in this group, 26/26 flaps on the sutured anastomoses side survived completely, whereas on the fibrinogen adhesive anastomoses side 1/26 flaps (R21) underwent partial necrosis and 1/26 flaps (R34) underwent complete necrosis.

Flap survival rates, after arterial anastomosis by each of the two techniques, did not show a dramatic statistical difference (paired t value 1.772, DF 45, $p<0.05$). The figures left the slight discrepancy between the two techniques open to interpretation. Certain technical factors about the experimental model were thought to slightly favour results in the suture anastomosis group. These factors are reviewed in the discussion section (see page 170), and the flap survival rate data should probably be interpreted as indicating that the two techniques did not differ.

Patency

In the 1 day follow up group, 9/10 sutured anastomoses were completely patent, and 9/10 fibrinogen adhesive anastomoses were completely patent.

In the 7 day follow up group, 10/10 sutured anastomoses were completely patent, and 10/10 fibrinogen adhesive anastomoses were completely patent.

In the 21 day follow up group, 25/26 sutured anastomoses were completely patent, whereas 23/26 fibrinogen adhesive anastomoses were completely patent.

Statistically, there was no significant difference between the patency rates of the two techniques (paired t value 1.354, DF 45, NS).

Technical findings

During the course of the experiment, as experience was gained with the adhesive anastomosis, a number of technical factors, that were important to the success of the adhesive anastomosis, became apparent.

Application of the microvascular clamps

It was important that the double approximating clamp was not applied too close to the cut vessel ends. If clamps were too close, when they were removed they lifted the glue away from the entrance to the sleeve, and this caused high pressure blood to leak out with the possibility of blowing the anastomosis apart.

Construction of the end-in-end anastomosis

The length of overlap in the sleeve was important. This was realised in the preliminary study and its importance became quite clear in the experimental study. A one diameter length of overlap was just satisfactory, but two diameters seemed safer.

End-in-end anastomoses should not be formed close to either branches or cauterised/clipped branch stumps. These posed little or no problem to sutured anastomoses, but for end-in-end anastomoses they could cause a number of things to go wrong:

1. A branch stump could be drawn into the anastomosis as the sleeve was being formed. This could produce a separation of the inner and outer walls and allow adhesive to flow into the lumen.
2. A branch stump could prevent the sleeve from being overlapped sufficiently, by getting caught up at the opening. This produced a short sleeve and, in consequence, it could either

increase the risk of the anastomosis being blown apart or increase the risk of allowing adhesive to flow into the lumen.

3. A branch stump could cause a poor interface between the adhesive and the outer margin of the sleeve, increasing the risk of an anastomotic blow out.

It was important to place the three stay sutures as evenly as possible, in order to be able to apply balanced tension to the anastomosis during application of the adhesive. Tension applied through the 180^{0} apart stay sutures corrected any tendency for the inner vessel to fold and increase the risk of adhesive flow into the lumen. At the same time that tension was applied to these two stay sutures, they could also be used to lift the anastomosis away from the surgical background material, thus improving adhesive coverage of the back wall. The back wall stay suture was important for ensuring that the back wall of the sleeve was well overlapped prior to the application of adhesive.

Application of the adhesive

A number of technical observations were made concerning the preparation and application of the fibrinogen adhesive:

1. It was essential to be meticulous in preparing the adhesive. This was not a difficult process but failure to pay attention to details in the manufacturers instructions could cause problems. It was particularly important to avoid the presence of bubbles in Duploject syringes. Bubbles resulted in uneven mixing and this could produce adhesive-free zones on the anastomotic wall with subsequent anastomotic leakage or even blow-out.

2. It was important to ensure that the back wall was adequately covered with adhesive. The technique to do this involved careful manipulation of the stay sutures while the adhesive was applied. Initially the surgeon dried the anastomosis whilst the

assistant prepared the Duploject syringe. Once ready to apply the adhesive, the surgeon, using fine forceps, held a side suture with one hand and the back wall suture with the other hand. The assistant held the other side suture in her non dominant hand and the Duploject in her dominant hand. The surgeon then rotated the vessel so that the back wall was visible, and the assistant started to slowly apply the adhesive. During the application, the surgeon rotated the vessel so that the assistant could continue to evenly apply the adhesive until the anastomosis was completely, uniformly and circumferentially covered. With a little practice this could be smoothly achieved in a single straightforward movement. It was essential to check the back wall of the anastomosis prior to application of the adhesive, as on a few occasions the inner vessel had, unnoticed, become unsleeved.

3. It was important to ensure that the anastomosis was uniformly covered with adhesive, both circumferentially and longitudinally for about two to three diameters proximally and distally. The outer surface of the anastomosis had to be as dry as possible to obtain an optimal interface with the fibrinogen adhesive. It was necessary to drop the adhesive from a height of one to two centimetres in order to produce a homogeneous mix. This technique also minimised the risk of potential problems arising from contact between either the anastomosis or the adhesive seal and the metal cannula of the Duploject. It was necessary to leave anastomoses a full three minutes, for cross linking to occur between the fibrin gamma chain and the gamma-gamma dimer, prior to releasing the microvascular clamps. Where possible, clamps should be placed distant to the anastomosis to avoid them becoming covered in adhesive and attached to the anastomosis. In the small operative field in this experimental model, this problem could not always be avoided, but if the adhesive was gently teased away from the clamp prior to removal, difficulties could be avoided.

It was essential to always remove the downstream clamp before the upstream clamp.

4. If an anastomosis had not obtained circumferential coverage with adhesive, or if the thickness of the adhesive layer was insufficient, leaks could occur when the clamps were released. In this situation a second application of adhesive to the weak area, prior to clamp release, successfully rectified the problem. If a leak occurred as the clamps were being removed, the application of gentle pressure, re-application of the clamp, and a further application of adhesive rectified the problem.

Subjective findings

An important subjective observation was that the adhesive technique was technically much easier to carry out than suturing, and also demanded less expertise and concentration from the surgeon.

Skin biopsies

The histological appearance of the skin biopsies exactly matched the clinical observations of flap appearance. Figure 17 illustrates the histological appearance of typical biopsies taken from areas where the skin was either clinically viable or not clinically viable. The main difference in the two sections is the global evidence of cell death in the biopsy from the non viable flap. There was no spectral variability of cell death. All specimens were either viable or non viable. Viability was always predictable from clinical observations, and the histological appearance of the skin biopsies did not provide any additional information.

Gross appearance of anastomoses

Figure 18 demonstrates typical features of the gross appearance of anastomoses after they had been removed at the time of sacrifice.

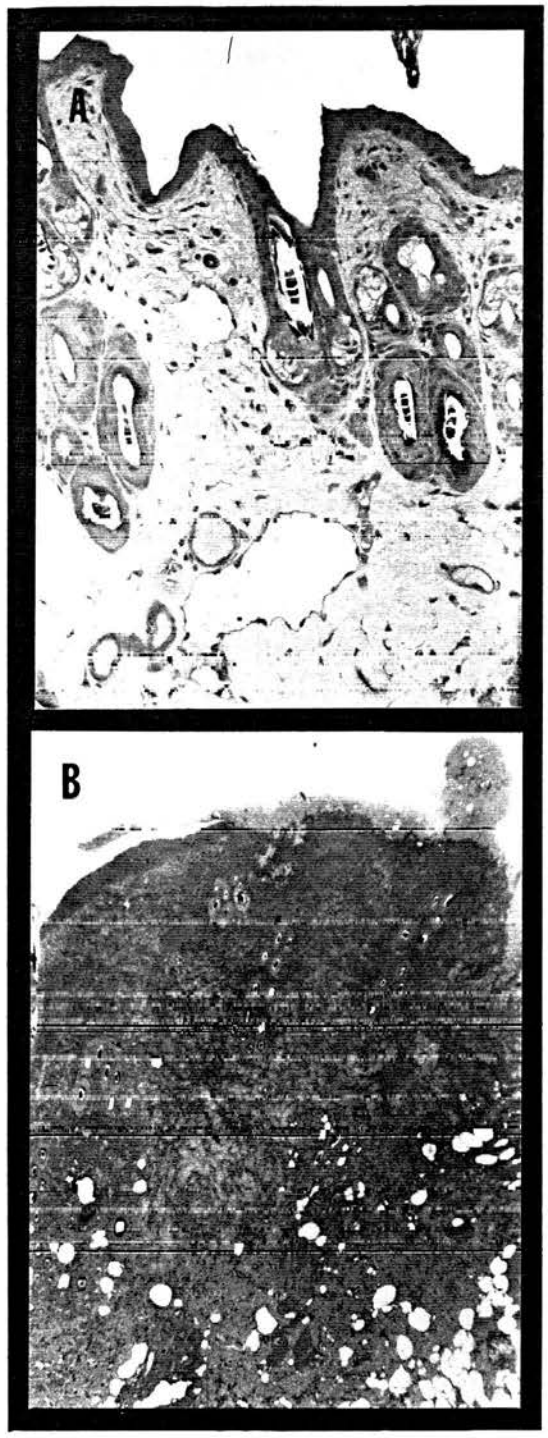

A
B

Figure 17. Photomicrographs of skin biopsy histological sections.

A Live skin (x31) demonstrating hair follicles and viable cells (R15).

B Dead skin (x31) demonstrating the absence of hair follicles and global cell death (R14).


A


B

Figure 18. Gross appearance of anastomoses at the time of sacrifice, shown to illustrate the sectioning of the anastomoses. Note the marking sutures, placed upstream to the anastomoses, to record the direction of flow. Photographs were taken through the operating microscope at x10 magnification.

A Fibrinogen adhesive anastomosis at day 7 (R13).

B Sutured anastomosis at day 7 (R12).

Most of the anastomoses were patent, and in those arteries, once the freshly formed clot had been gently washed away, it was possible to identify and observe the anastomotic region. In the small number of partly and completely thrombosed anastomoses, the lumen was filled with a plug of blotchy dark red/greyish thrombus, and it was difficult to see exactly where the anastomosis was located, particularly in the twenty-one day specimens. There were not enough failures to comment on similarities and differences between the two types of anastomosis, but they did not appear to differ greatly.

Sutured anastomoses

One day group. Patent anastomoses were easy to locate, and the lumen contained minimal quantities of pinkish fibrinous material adherent to the vessel wall at and around the anastomotic site. The cut vessel ends were still sharply demarcated, but were held together by the sutures, which were easily visible, and usually appeared to be evenly spaced. Outside the vessel wall a small amount of haematoma was frequently seen.

Seven day group. Patent anastomoses were still easy to locate. There was little or no intraluminal debris. Sutures were still visible, but the cut vessel ends appeared to be blending together with some tissue adhesion. Little or no extra-mural haematoma was visible, and scar was beginning to form.

Twenty-one day group. It was much harder to find the exact location of the anastomoses, although this could usually be located from the position of extra-mural parts of the sutures. The lumen was clear of debris, and sutures were difficult to see on the lumenal surface. The cut vessel ends were firmly adherent with scar, which was also abundant outside the anastomoses.

Fibrinogen adhesive anastomoses

One day group. Patent anastomoses were easy to locate, and the lumen contained a small amount of pinkish, fibrinous material adherent to the vessel wall at and around the inner margin of the sleeve. This intra-lumenal material did not appear to differ from that seen in the sutured anastomoses. The sleeves were easily visible, and, although there was some adhesion between the inner and outer walls, they were not firmly adherent. Anastomoses seemed to be held together by the stay sutures and the white fibrinous material (presumably the fibrinogen adhesive) outside the anastomoses. There was no evidence that any stay suture had been placed into the lumen. Some haematoma was usually present around the outside of the anastomosis.

Seven day group. Patent anastomoses were still easy to locate. There was little or no intra-lumenal debris. The inner and outer layers of the sleeves were still clearly visible, although they were becoming more adherent. The inner margin of the sleeves was still quite well demarcated. Stay sutures could still be located and there was no evidence of intra-lumenal insertion of any stay sutures. Fibrinogen adhesive was no longer visible outside the anastomosis, where scar was beginning to form.

Twenty-one day group. It was much harder to find the exact location of the anastomoses, although they could still be located because of the extra-mural parts of the stay sutures. The lumen was clear of debris, and the inner margin of the sleeves was usually only visible by a smooth transverse irregularity in the lumenal surface. The two walls of the sleeves were usually firmly adherent with scar, which was also abundant outside the anastomoses.

Histology of anastomoses

Histological sections of both experimental adhesive and control sutured anastomoses are illustrated in Figure 19 (1 day), Figure 20 (7 day) and Figure 21 (21 day).

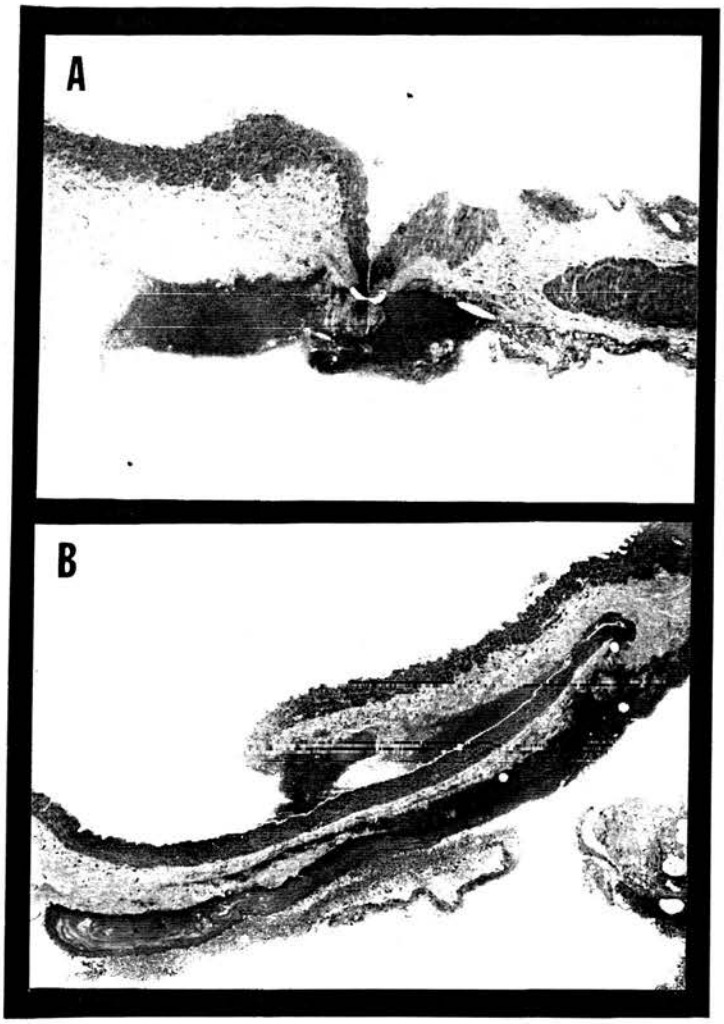

A
B

Figure 19. Photomicrographs of the two types of anastomosis at 1 day.

A Sutured anastomosis (x31).

B Adhesive anastomosis (x31).

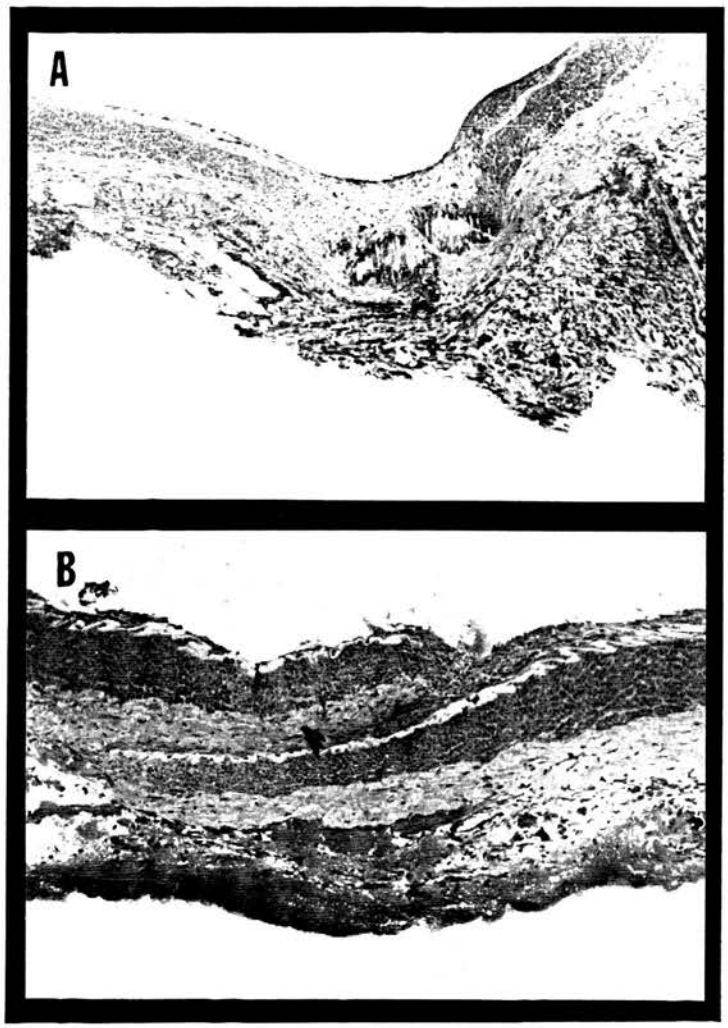

A
B

Figure 20. Photomicrographs of the two types of anastomosis at 7 days.

A Sutured anastomosis (x50).

B Adhesive anastomosis (x50).

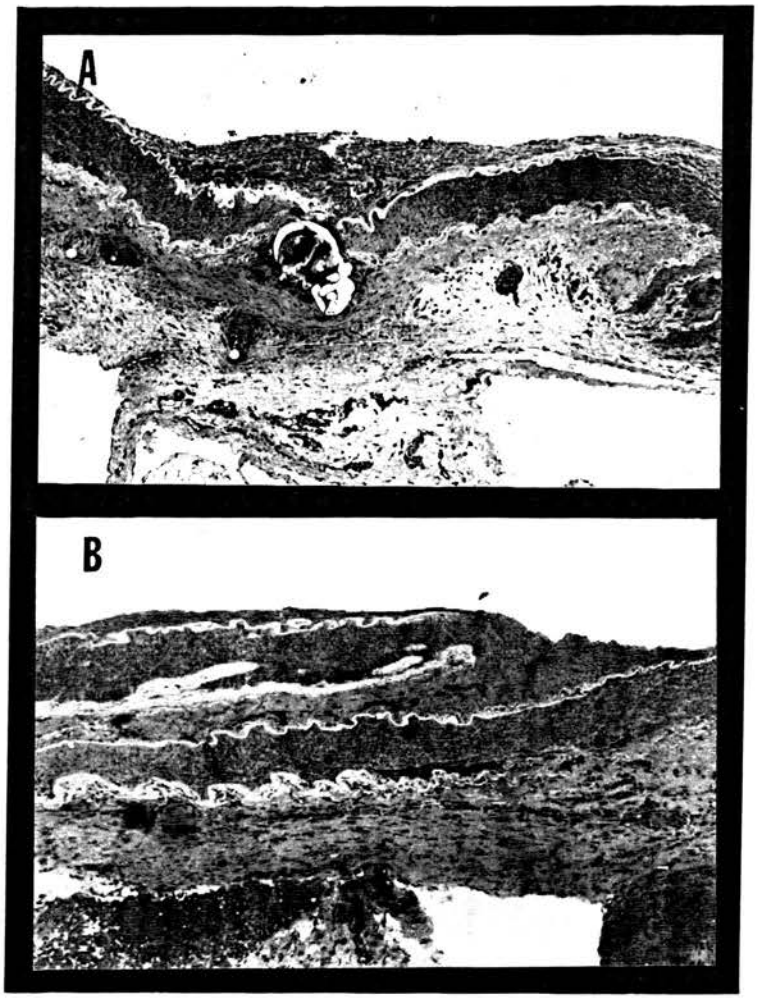

A
B

Figure 21. Photomicrographs of the two types of anastomosis at 21 days.

A Sutured anastomosis (x50).

B Adhesive anastomosis (x50).

Sutured anastomoses

One day group. Figure 22 illustrates details of a typical sutured anastomosis at 1 day.

The lumen was dilated in some of the specimens. At the site of anastomosis suture material was visible in many of the specimens, and the vessel walls were slightly bunched. In most specimens small amounts of clot, consisting of platelets, blood cells and fibrin, were present at the anastomotic site. Occasionally the endothelium was broken, but more often it was intact and remained one to two cells thick. The media thinned out towards sutures, and tissue in the vicinity of the knot was undergoing hyaline degeneration with loss of cell structure and no visible cell nuclei. The media remained intact right up to the sutures, as did the internal elastic lamina. In cases where the section had been cut between sutures, media was present and there was no cellular destruction. The adventitia, similarly, was not damaged except where it was strangled by a knot. In locations where the adventitia had been sutured it had lost its cellular appearance and only few nuclei were present, although elastic and collagen fibres still remained visible. Outside the anastomosis haematoma was seen, and this consisted mostly of red cells and the occasional white cell.

At 1 day there was almost no reaction to suture material (Figure 23).

Seven day group. The lumen was almost free of clot, and its surface was covered with endothelial cells. At the anastomotic site there was intimal hyperplasia (Figure 24) and this tapered away from the anastomosis in a smooth manner. The hyperplastic cells were arranged longitudinally, and resembled fibroblasts. The internal elastic lamina was deep to the intimal hyperplasia, and remained adherent to the media, although in some sections it appeared to be layered and fragmented. The media and adventitia were undergoing hyaline degeneration, but the extent of this varied

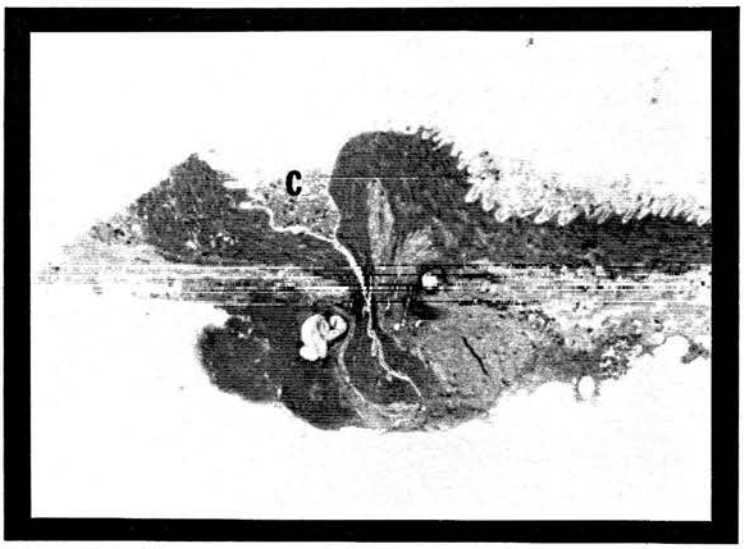

C

Figure 22. Details of sutured anastomosis at 1 day (x50). The lumen is at the top of the illustration. There is a small amount of clot (c) at the anastomotic site. Thinned media and adventitia are entrapped in the knot.

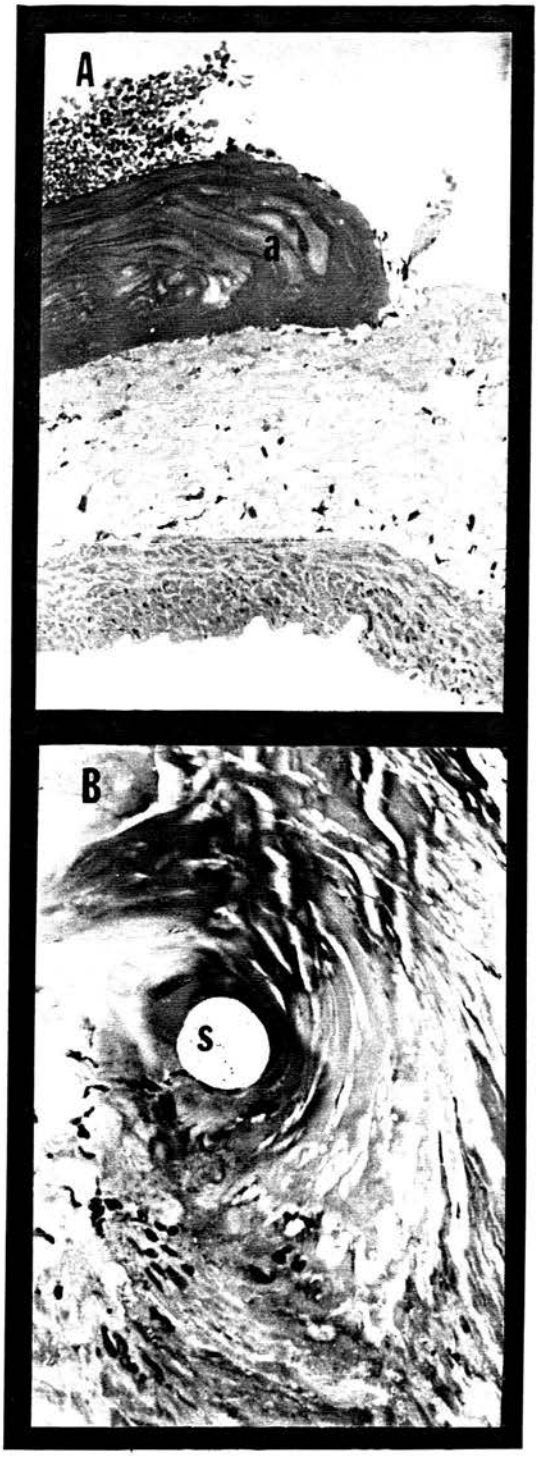

A
a
B
S

Figure 23. Reaction to fixation technique at 1 day.

A Adhesive (x125). The lumen is at the bottom of the illustration. Adhesive (a) can be seen outside the vessel wall and, apart from some red blood cells and a few polymorphonuclear leucocytes, there is very little tissue reaction.

B Suture (x312). There is some hyaline degeneration adjacent to the suture (s), but there is very little tissue reaction.

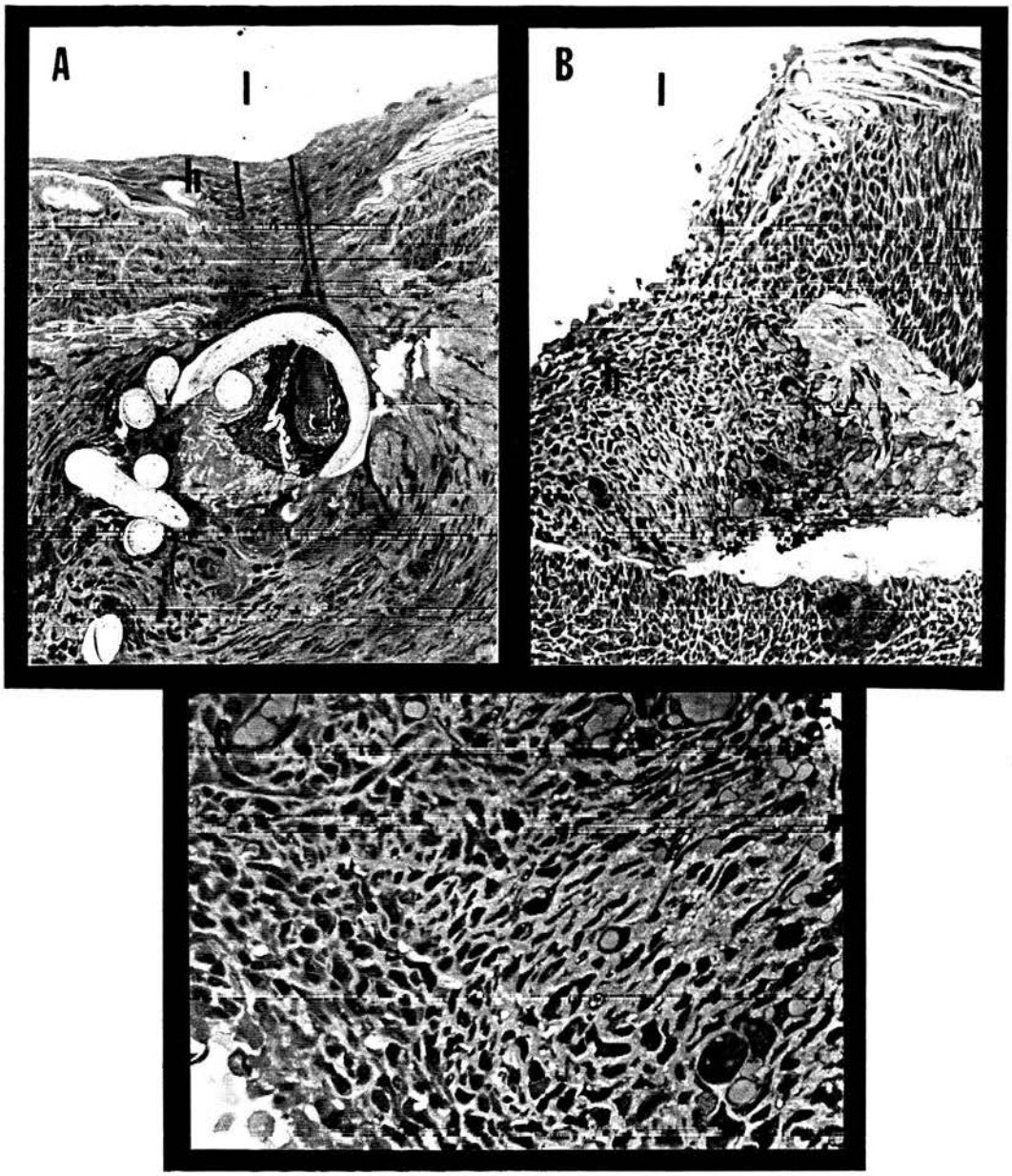

A
I
II
B
I
II

Figure 24. Details of hyperplastic intimal cells (h) at 7 days. The lumen is labelled (l).

A Sutured anastomosis (x125).

B Adhesive anastomosis (x125).

C Details of the cells (x312).

depending on the plain of histological section. The media narrowed towards knots, and in areas of hyaline degeneration there was loss of cell structure with only a few remaining cell nuclei. In the adventitia overlying sutures there was a fibroblastic reaction, which appeared to represent organisation of the extra-vascular haematoma. Sutures were surrounded by a moderate inflammatory response which contained giant cells (Figure 25).

Twenty-one day group. There was usually no intramural debris. The endothelial layer was completely reconstituted, and intimal hyperplasia (Figure 26) was still very visible. The hyaline necrosis at the knots was still apparent, but the media was normal up to that point. Folds in the internal elastic lamina were still present. The inflammatory reaction around suture material was diminished (Figure 27), although some mononuclear cells were still to be found in the region of sutures. There was no visible haematoma outside the anastomoses. This had been replaced by fibrous tissue.

Fibrinogen adhesive anastomoses

One day group. Figure 28 illustrates details of a typical adhesive anastomosis at 1 day.

The inner and outer layers of the sleeve could be clearly identified. The inner vessel wall was separated from the outer vessel wall by a clot consisting of platelets, fibrin, and some blood constituents. External to the outside sleeve wall, fibrinogen adhesive was visible. In some cases, a small amount of this had found its way between the two layers of the sleeve, but this was never a large quantity and it did not travel as far as the lumen in any specimen. In some sections, red blood cells had leaked through the sleeve, but in almost all cases the fibrinogen adhesive had kept them within the sleeve and prevented extra-vascular bleeding. On the lumenal side, the intima was very similar in appearance to the sutured anastomoses, and tended to remain one to two cells thick.


A
I
S
B
S

Figure 25. Reaction to suture (s) at 7 days. The lumen is labelled (l). The giant cell reaction is clearly seen.

A Low power (x125).

B High power (x500).

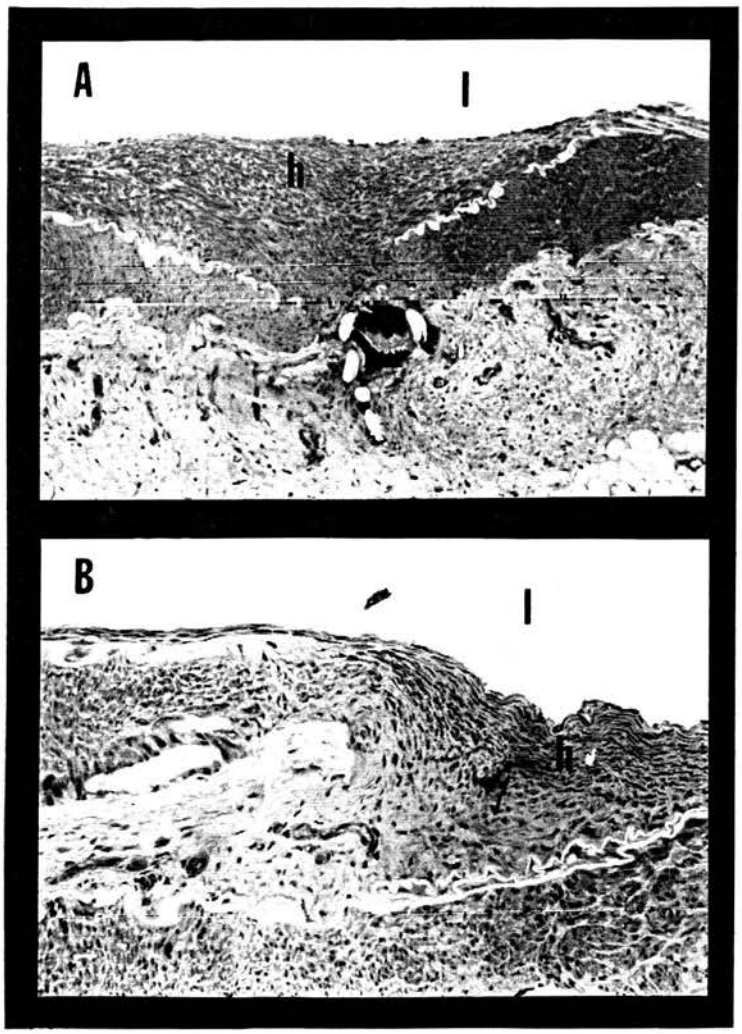

A
l
h
B
l
h

Figure 26. Details of hyperplastic intimal cells (h) at 21 days. The lumen is labelled (l).

A Sutured anastomosis (x50).

B Adhesive anastomosis (x125).

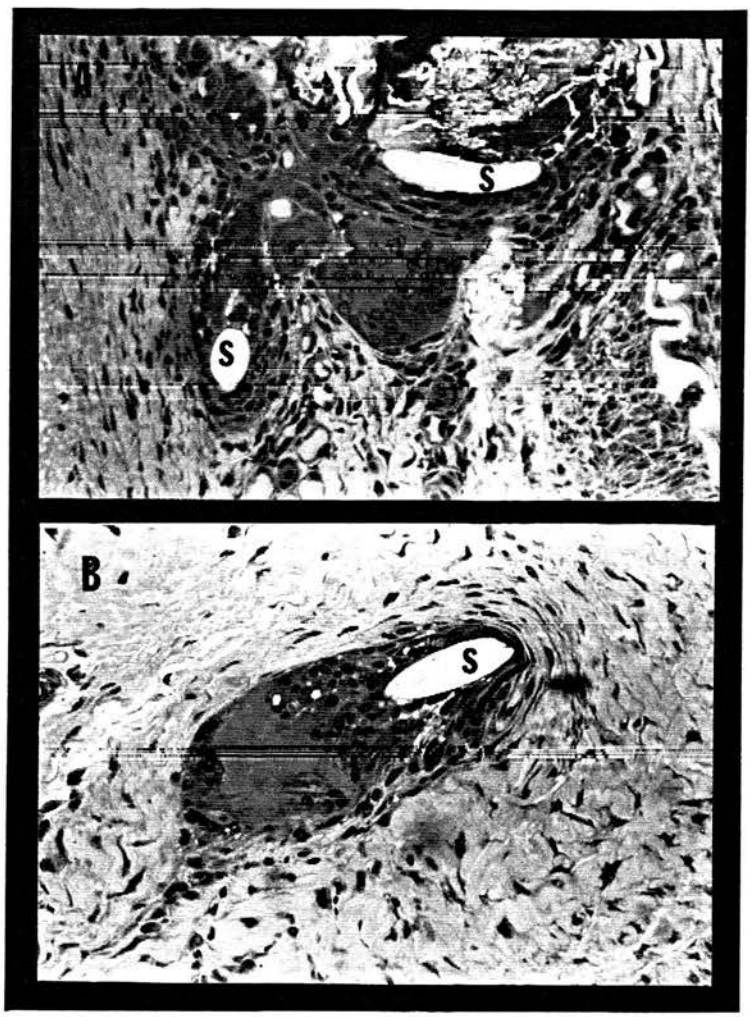

A
S
S
B
S

Figure 27. Reaction to suture (s) at 21 days. The giant cell reaction was still present.

A At the knot (x200).

B Single strand (x200).

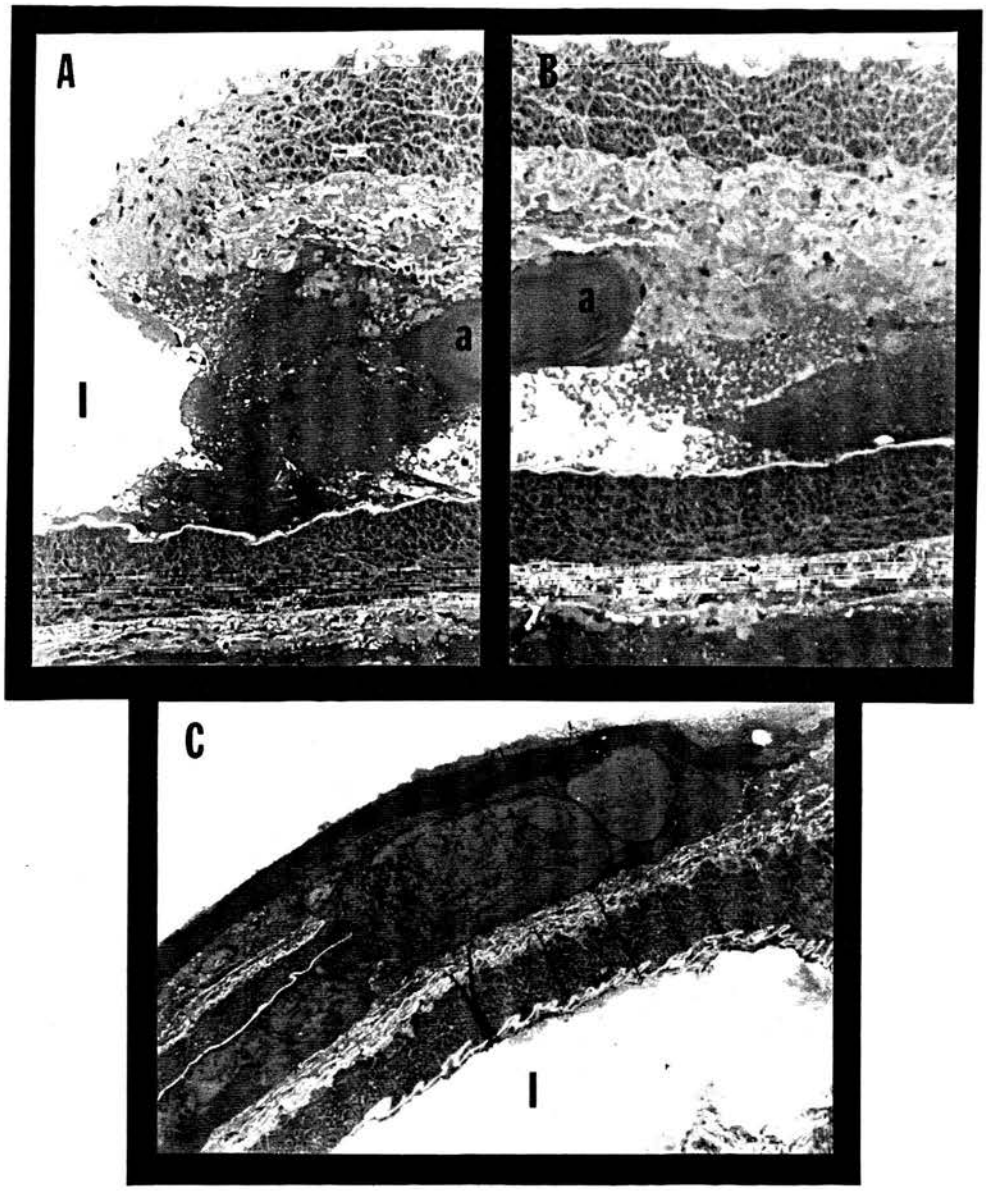

A
a
l
B
a
C
l

Figure 28. Details of adhesive anastomosis at 1 day. The lumen is labelled (l) and adhesive is labelled (a).

A Free inner margin of the anastomosis (x125).

B Contents of end-in-end anastomosis (x125).

C Outer margin of anastomosis demonstrating containment of haematoma by adhesive (x50).

The media and adventitia appeared normal in appearance, and there were no signs of necrosis or hyaline degeneration. In some of the specimens, stay sutures could be seen near the outer entrance of the sleeve. There was no inflammatory reaction to the fibrinogen adhesive (Figure 23), although a few polymorphonuclear leucocytes were seen in some specimens. Some of the sleeves had come apart during processing but this was thought to be artifactual.

Seven day group. The sleeves were still easily identified. There was very little debris in the lumen, although some red and white cells were still adherent to the endothelium. Intimal hyperplasia was seen with cells appearing to stream across the entrance of the sleeve towards the intima of the outer vessel wall. The hyperplastic cells were similar in appearance to those in the sutured anastomoses (Figure 24). Some specimens gave the impression of a superficial layer of streaming overlying a layer of cells that were orientated as if they had come out of the media. There was no necrosis in the media or adventitia. The material in the sleeve itself was organising. Fibrinogen adhesive was present in much smaller quantities than in the one day specimens, and was always surrounded by a marked acute inflammatory reaction (Figure 29), consisting almost exclusively of polymorphonuclear leucocytes, with a few chronic inflammatory cells. In a number of places, the inflammatory cells could be seen invading and eroding the fibrinogen adhesive. Small areas of sequestered adhesive were present, and these were probably islands not yet destroyed by the acute inflammatory reaction.

Twenty-one day group. There was almost no intracellular debris. Intimal hyperplasia had smoothed over the mouths of the sleeves and was covered by a layer of endothelial cells. The cellular components of the intimal hyperplasia cells looked similar to that in the seven day specimens, with cells streaming between the two vessel wall layers (Figure 26). The contents of the sleeve itself were becoming well organised into fibrous tissue, although the

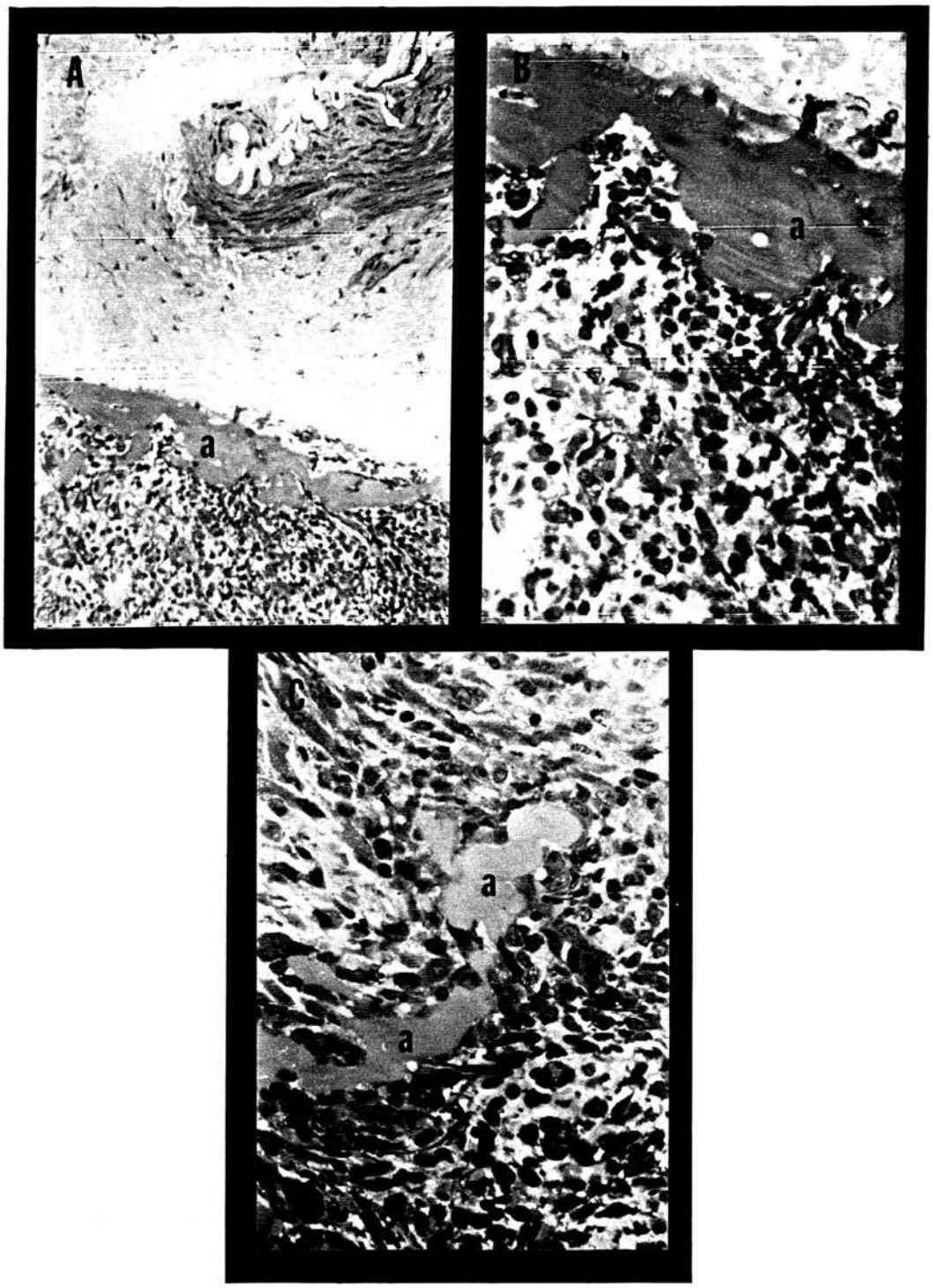

A
a
B
a
C
a
a

Figure 29. Reaction to adhesive (a) at 7 days. A marked acute inflammatory reaction surrounded the adhesive.

A Low power (x125).

B High power (x312).

C Sequestration of adhesive (x312).

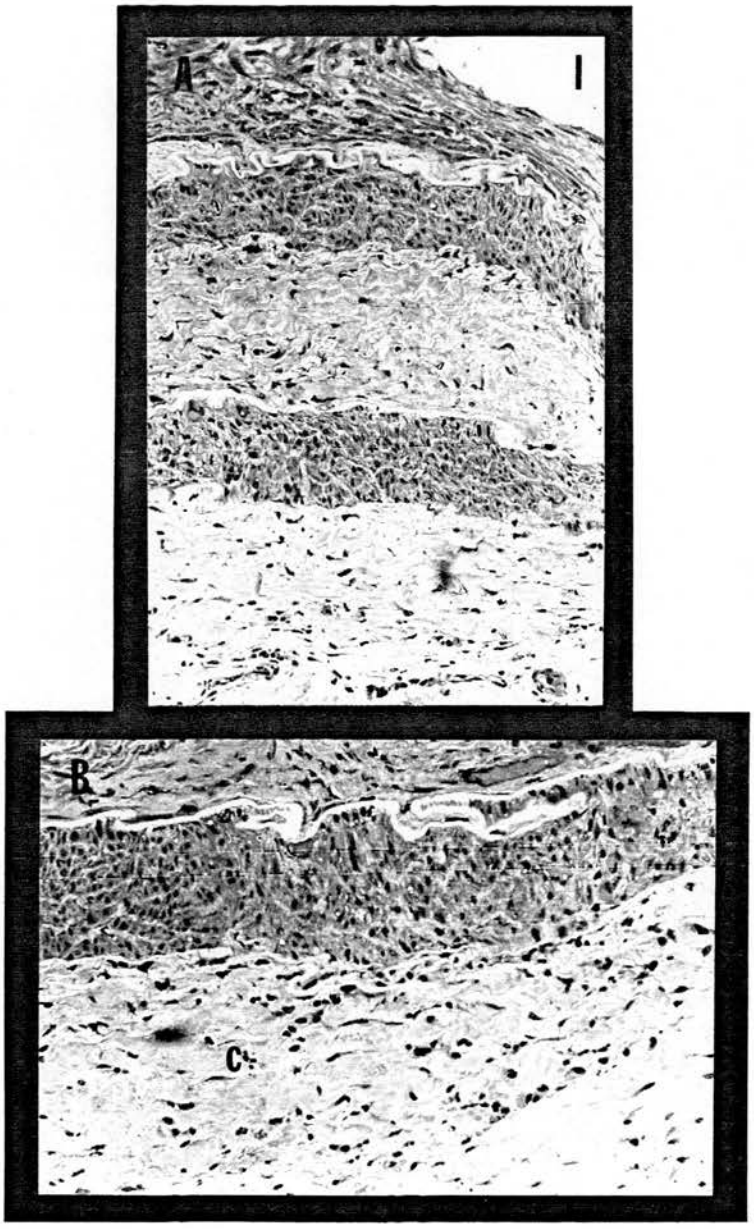

A
B
C

Figure 30. Reaction to adhesive at 21 days. The adhesive was no longer visible and the acute inflammatory response to it had completely settled, although a few chronic inflammatory cells (c) were still visible at the periphery. The lumen is labelled (l).

A Low power (x125).

B High power (x160).

inner and outer vessel walls were still clearly visible throughout the sleeve. There was no sign of any fibrinogen adhesive. The outer margin of the sleeve was sealed by fibrosis. The acute inflammatory response to the adhesive had completely settled (Figure 30), although a few chronic inflammatory cells still remained at the periphery.

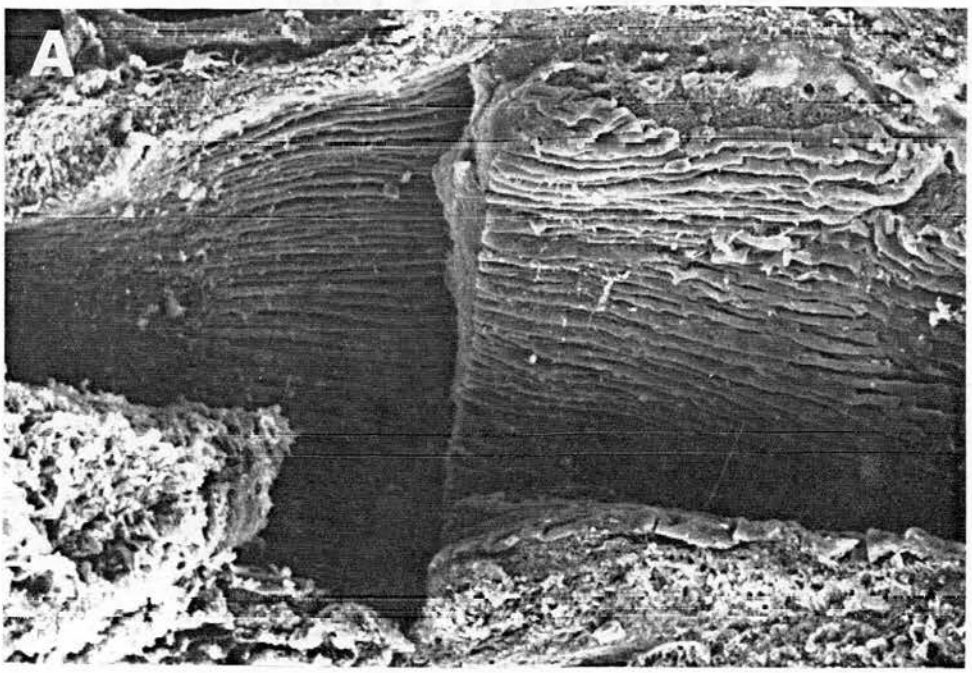
Scanning electron microscopy of anastomoses

Scanning electron micrographs of both experimental adhesive and control sutured anastomoses are illustrated in Figure 31 (1 day), Figure 32 (7 day) and Figure 33 (21 day).

Sutured anastomoses

One day group. Although some of the specimens contained a lot of intra-lumenal debris, most of the anastomoses could be well visualised. The intimal endothelial layer was thrown up into longitudinal folds indicating vessel wall contracture during processing. Sutures could be seen and, where they perforated the vessel wall, endothelial cells were torn and there was some mural thrombus formation. In places, mural thrombus was adherent to the sutures themselves, but this was not universal. There was de-endothelialisation at the site of vascular clamp application, and these locations were covered by a thin layer of leukocytes and platelets. Mural thrombus was present at the anastomotic site, and red and white blood cells were adherent to the adjacent endothelium.

Seven day group. The endothelium had nearly reconstituted over the surface of the anastomoses, although not all sutures were covered by endothelial cells. Some breaks in the endothelial lining were still visible at the anastomotic site, and patches of mural thrombosis still remained. In some areas, occasional blood elements were attached to the endothelial surface. Compared to


A

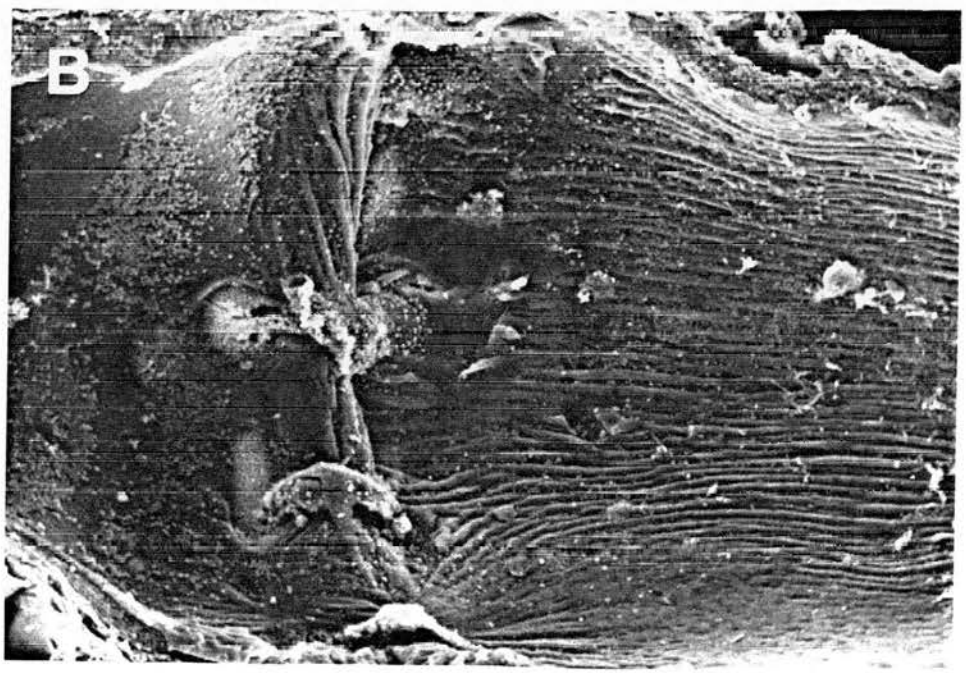
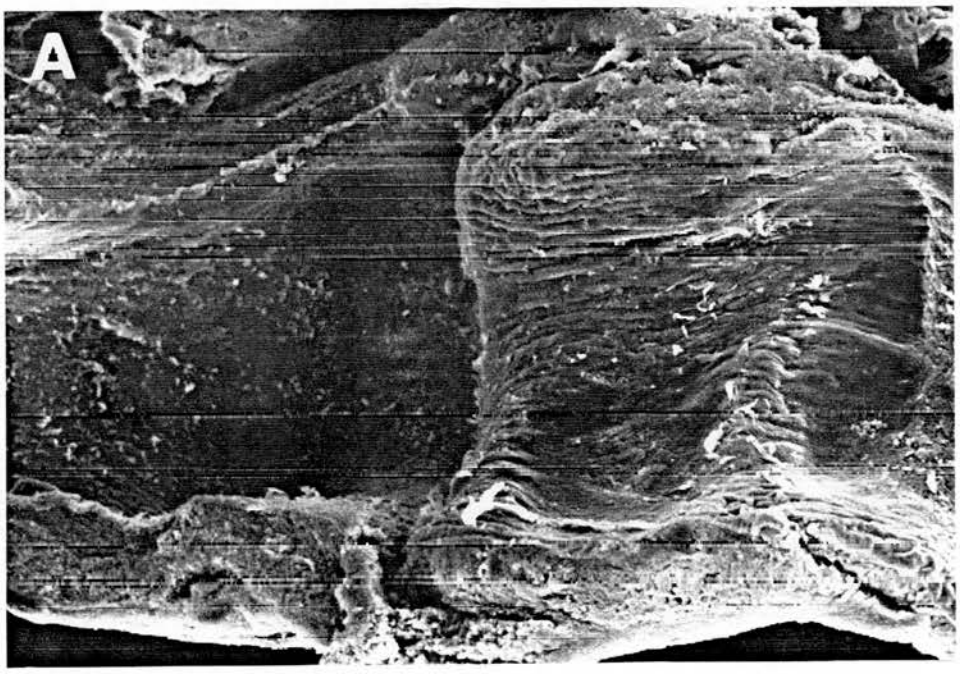

B

Figure 31. Ultrastructure at 1 day. Low power views of the lumenal surface of experimental and control anastomoses.

A Adhesive anastomosis (x55) showing the inner wall of the sleeve on the right. Note the small amount of non-propagating clot at the inner margin of the end-in-end anastomosis.

B Sutured anastomosis (x70) showing mural thrombus at the needle puncture sites.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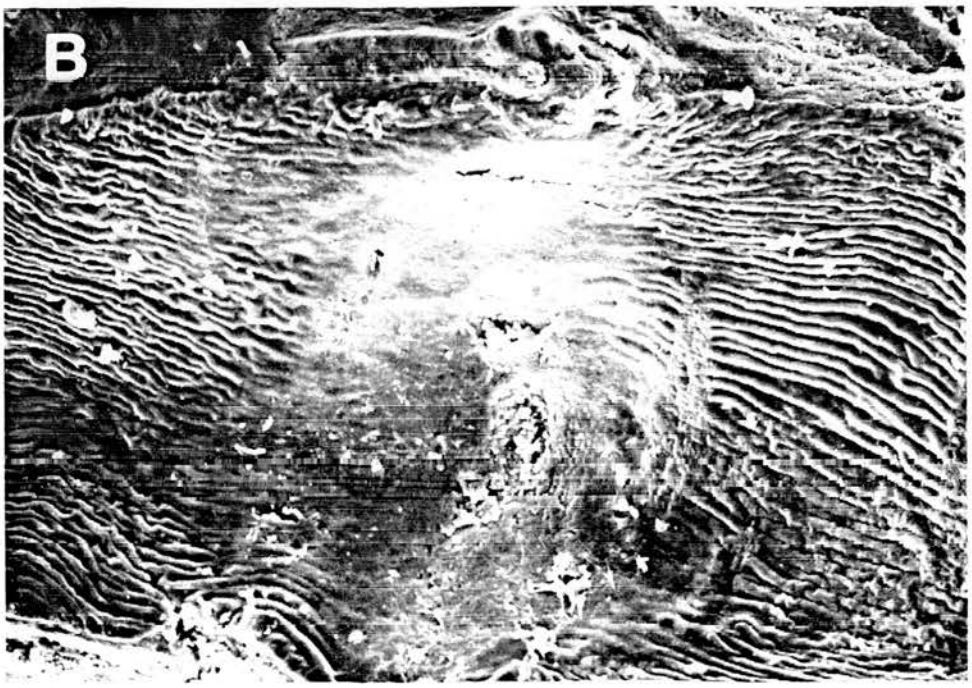
B

Figure 32. Ultrastructure at 7 days. Low power views of the lumenal surface of experimental and control anastomoses.

A Adhesive anastomosis (x55) showing the appearance of the hyperplastic intimal cells to the left of the inner margin of the end-in-end anastomosis (at the centre of the illustration).

B Sutured anastomosis (x70) showing that the hyperplastic intimal cells (in the centre of the illustration) cover the anastomosis and the sutures, which can no longer be seen).

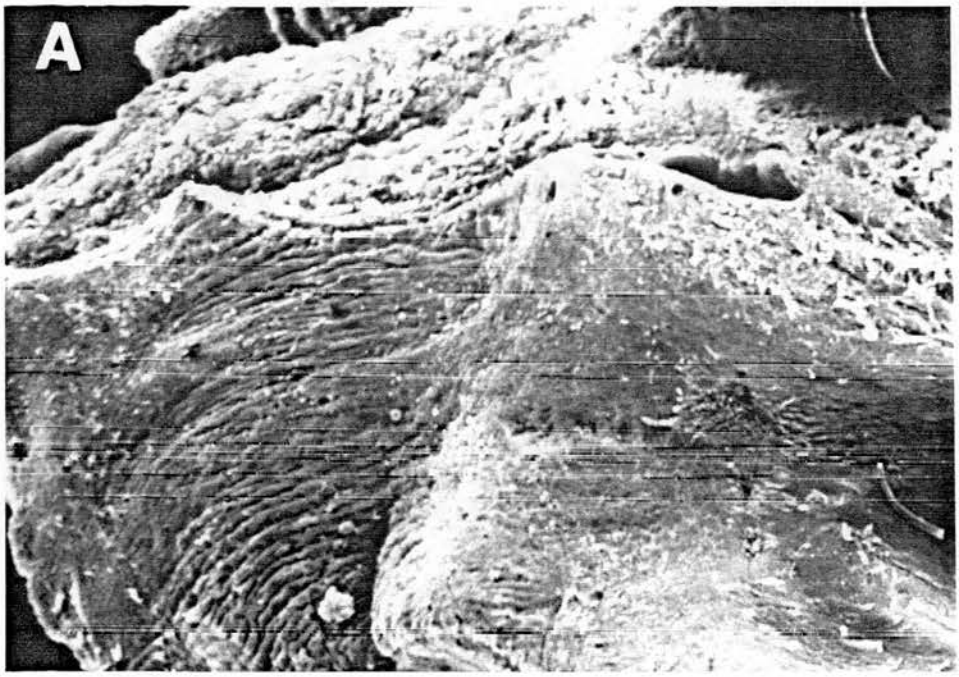
A

Figure 33. Ultrastructure at 21 days. Low power views of the lumenal surface of experimental and control anastomoses.

A Adhesive anastomosis (x55) showing that the lumen is re-endothelialised and its contour is much smoother.

B Sutured anastomosis (x70) which is no longer visible from the lumenal side.

one day specimens, there was a tendency towards loss of thrombosis and regeneration of endothelium.

Twenty-one day group. There was almost no intra-lumenal debris. The endothelium was completely reconstituted and bridged the anastomoses. In many specimens it was difficult to identify where the anastomosis was located. In most cases the sutures were covered by endothelium, but their location could sometimes be determined because of contour variation. Mural thrombus was rare, and the lumenal surface appeared smooth with endothelial cells orientated longitudinally.

Fibrinogen adhesive anastomoses

One day group. The two layers of the sleeve were seen with sharp demarcation at the inner margin. Endothelial damage and desquamation was seen at the site of vascular clamp application, and blood cells were adherent in that region. The endothelial lining appeared normal right up to the margin of the sleeve, although there were a small number of adherent blood cells. Some specimens demonstrated thrombus at the mouth of the sleeve, but none showed propagation of this distally.

Seven day group. There was still demarcation at the mouth of the sleeve although the free margin was becoming smoother as endothelial cells regenerated. Thrombosis was still visible at the mouth of the sleeve, but there was never any distal propagation. In areas where vascular clamps had been applied, regenerating endothelial cells were seen alongside mural thrombus. In general, the endothelium was free of debris but adhesion of occasional blood elements was widespread.

Twenty-one day group. It was possible to identify the inner layer of the sleeve only by a contour defect. Apart from this, the inner layer of the vessel was smooth and covered with endothelial cells which looked fairly normal in appearance and were oriented in the direction of flow. Clamp injuries were no longer visible, and

the intima was smooth with almost no adherent debris or blood elements.

DISCUSSION

This study was no different to any other laboratory experiment in that it contained strengths and weaknesses.

The study was generally believed to be a strong one in that:

1. There was opportunity to thoroughly research the initial idea, to develop it in a preliminary study, and then to test it in an experimental model.
2. The experimental study was designed to be consecutive, controlled and prospective.
3. It was possible to study anastomotic success or failure by a variety of different methods.

There were, however, weaknesses. In particular it should be noted that:

1. The small operative field in the rabbit groin flap model resulted in a number of minor technical differences between the adhesive and sutured anastomoses.
2. The results of this animal experiment, in common with other animal experiments, might not be directly applicable to human situations.

The experiment is relevant to contemporary Microvascular Surgery because:

1. It adds a new anastomotic technique to the current armamentarium.
2. It adds information to the basic knowledge concerning the healing process of microvascular anastomoses.

Discussion of the results has been divided into three sections: experimental aspects, technical aspects, and biological aspects.

Experimental aspects

Comparison of anastomotic techniques

According to the Daniel and Terzis[86] criteria of an ideal adhesive substance (see page 72), the fibrinogen adhesive, Tisseel, would appear to be a very satisfactory material. It has been demonstrated to be superior to the cyanoacrylics[315], and in many respects is close to the ideal adhesive.

Sully et al[364] suggested that it is necessary to demonstrate a number of specific advantages "if a seemingly easier and more reliable technique for microvascular repair is to be advocated:

1. Speed of completion of anastomosis.
2. Less intra-lumenal suture exposure.
3. Less vessel trauma owing to fewer sutures and less manipulation of vessel ends.
4. Prevention of intimal separation.
5. Convenient matching of different sizes of donor and recipient vessels."

It is true that it is necessary to demonstrate superiority in these areas, but other factors are also important. According to my own criteria for suggesting that one type of anastomosis has advantages over another it would be necessary to:

1. Carry out a controlled comparative study.
2. Ensure that microvascular patency and flap survival are, statistically as good as, or better than, the 'gold standard' conventional suture technique.
3. Demonstrate specific, and worthwhile, areas in which it excels.
4. Demonstrate that it does not have any detrimental side effects.

This study is the only consecutive, controlled, prospective trial that has been undertaken to compare a fibrinogen adhesive technique with the 'gold standard' conventional suture technique. The numbers were sufficiently large for statistical analysis.

The rabbit bilateral femoral artery/groin flap model was satisfactory for the experiment. It enabled the two anastomotic techniques to be compared in a very controlled manner. It also provided a large number of parameters for comparison. Its main drawbacks were that:

1. The operative field was small.
2. The presence of numerous branches in the region of the anastomoses sometimes produced technical difficulties, which resulted in an unfavourable bias against the end-in-end technique.

Results indicated that the technique, developed in the preliminary study, was reliable in its ability to produce satisfactory microvascular end-in-end anastomoses. The adhesive end-in-end anastomoses were statistically faster and easier to complete than sutured end-to-end anastomoses. The overall anastomotic patency rate compared favourably with previous reports in the literature.

Interpretation of data

It was necessary to look carefully at the interpretation of the data. Although there was no statistical difference in the patency and flap survival rates between the two techniques, the adhesive technique slightly predominated in the small number of failures. It may be that this occurred by chance alone, but the possibility that the adhesive technique was less satisfactory must also be examined.

It is likely that if the technique was basically faulty, the results would have been poor. At best, there would have been a spectrum ranging from complete vascular occlusion, through varying degrees of intra-lumenal thrombus formation, to a small number of satisfactory results. In addition, it would be reasonable to expect that a possible cause of failure (e.g. intra-lumenal flow of adhesive or excessive inflammatory response) might have been found.

There was no spectrum. The vast majority of anastomoses were successful, and very comparable to their sutured controls. There were only a small number of failures, and these were also comparable to their sutured controls.

Further investigation revealed that it was more likely that the technique was satisfactory, but problems with the model accounted for the slightly greater number of adhesive failures. A review of the detailed operative notes, that were kept on each animal, indicated that arterial branches located close to the anastomoses may not have been handled in an ideal manner in some of the procedures, particularly early in the series (see page 139). Operative notes indicated that one, and likely two, failures involved branches being drawn too close to, or actually into, sleeves. This technical error could explain the statistically insignificant, sporadic predominance of failures in the adhesive anastomoses. This problem arose because of the small operative field, the short length of vessel available for anastomosis, and the inability to avoid the close proximity of cauterised or clipped side branches. These close side

branches would have little or no effect on the sutured end-to-end technique, but would, if unrecognised, be detrimental to the adhesive end-in-end technique. The problem would be unlikely to occur in human clinical situations, where the operative field is larger, and there is usually a much greater length of vessel available for anastomosis. Later in the series, when the phenomenon had been recognised, it was found that, in the small number where it occurred, it was usually possible to make a satisfactory end-in-end anastomosis. When this was not possible, the procedure was aborted and the animal replaced.

Anastomotic time

The time taken to complete a fibrinogen adhesive anastomosis was shown to be significantly less than that necessary for a sutured anastomosis. The real time saved for a single anastomosis, however, was only a few minutes. These few minutes were demonstrated to significantly reduce the total operating time in the uncomplicated experimental model. It is unlikely, however, that these few minutes would make a great deal of difference to the total operating time in a human clinical operation, where most of the time is spent handling problems related to donor and/or recipient site preparation.

Subjective observations

The subjective observation, that adhesive anastomoses were technically much easier to carry out than suturing and required less expertise and concentration from the surgeon, might, in a clinical situation, provide the most important indication for using this new adhesive technique (see page 187).

Technical aspects

Intra-operative technical failures

Intra-operative technical failures occurred in both sutured and adhesive anastomoses in almost equal numbers. According to the experimental protocol these were revised when they occurred in end-to-end sutured anastomoses, and animals were replaced when they occurred in end-in-end adhesive anastomoses. Clinically, it is mandatory to revise or replace anastomoses that are judged to be faulty at the time of operation. The small number of occasions that this was necessary would be fairly typical of that encountered in standard clinical practice. The nearly equal distribution between sutured and adhesive intra-operative failures suggests that the protocol did not impart any bias on the overall results.

Technical differences between the two types of anastomoses

Despite the attempt to develop the perfect model for a completely controlled, comparative study, there were still some differences in the technical aspects of the two types of anastomosis. In a small number of cases it was felt that these experimental differences may have had an effect on the results. In other cases they influenced the way in which decisions were made in problem solving situations.

In adhesive anastomoses there was inevitable shortening of the vessel due to the end-in-end technique used. This was not present in the end-to-end sutured anastomoses. This created a small difference between the experimental and control sides. In some animals there was a redundancy in vein length on the adhesive side. In a human clinical situation this could be corrected by either shortening the vein or by allowing it to adopt a smooth curve and 'take up the slack'. The limited space available in the model did not allow this to be done and it may be that this increased the risk of kinking with the possibility of developing a

primary venous and secondary arterial failure. It is thought that this phenomenon probably accounted for the problems in one rabbit (R5) and may, possibly, have contributed to problems in two others (R21; R34). If failures occurred as a result of this problem, they should be considered to be related to the experimental model and not to the fibrinogen adhesive anastomotic technique. Unfortunately there is no way of knowing which anastomoses were affected in this way, but it should be noted that this complication was biased, unfavourably, towards the adhesive anastomoses.

The differences between the two types of anastomosis also influenced the way decisions were made if an anastomosis was thought to be unsatisfactory intra-operatively. End-to-end sutured anastomoses could be excised and revised with minimal resection of vessel. This did not appear to be associated with redundancy in venous length or other problems. In the case of end-in-end adhesive anastomoses, however, it was not possible to simply excise and revise problem anastomoses. The reason for this was related to the short length of vessels available in this experimental model. If an end-in-end anastomosis was resected there was not enough artery left to create another end-in-end anastomosis. Even if it had been possible, it would have substantially increased the problems with venous kinking, and, due to the small operative field, might have been associated with increased risk for entry of adhesive into the lumen. It was felt that these unfavourable factors could exert an unfair bias towards the adhesive anastomoses, and that this would not occur in the human clinical situation. In human free tissue transfer procedures, there would usually be enough vessel available to resect and revise a failed end-in-end anastomosis. If this was not possible, the anastomosis would be replaced with a reversed vein graft. The large operative fields would allow for removal of excess adhesive, and re-positioning of vessels to avoid kinking. For these reasons, when intra-operative failures occurred on the adhesive

side, it was felt reasonable to abandon the procedure, and start again with a fresh animal.

Technical factors relating to end-in-end anastomoses

In the preliminary study it was noticed that, when the microvascular clamps were released, and high pressure blood was allowed into the lumen of an end-in-end anastomosis, the sleeve shortened and the cut edge of the inner vessel appeared as a curved transverse line. It was assumed that this indicated the effect of intra-lumenal pressure trying to push the components of the anastomosis apart. Frequently 'unglued' end-in-end anastomoses did blow apart and this is accounts for most of the difficulties that microvascular surgeons have had in reproducing Lauritzen's[204-209] success with the sleeve technique. Lauritzen[204] did not stipulate how much overlap was ideal or necessary for satisfactory sleeves, and did not describe any blow-out failures. He did write that the proximal vessel should be held two millimetres from the cut end, suggesting that his sleeves were one to one and a half diameters long. Johnston[177] observed microvascular surgeons using end-in-end anastomoses in China, and commented that the sleeves were long, and the overlap appeared to be at least two, and sometimes more, vessel diameters in length.

In the preliminary study it was discovered that when Tisseel was applied to the surface of end-in-end anastomoses, blow-outs did not occur. In the experimental series this finding was confirmed and predictably patent anastomoses resulted. Release of the arterial microvascular clamps was associated with some sleeve shortening, but the Tisseel held the anastomoses together and prevented extra-vascular leakage of blood. Thus the technical function of Tisseel in the anastomoses appeared to be partly adhesive and partly sealant.

It is known that if the fibrinogen adhesive had flowed through the sleeves and into the lumen, there would have been a high

incidence of anastomotic failure. This did not occur and the histological specimens demonstrated that only the outer part of the sleeves contained any fibrinogen adhesive. Adhesive flow towards the lumen was probably prevented by surface tension between the two vessels being anastomosed. Two technical factors were important if separation of the two vessel walls was to be avoided:

1. Stumps of side branches, cauterised or clipped, should not be pulled into the sleeve.
2. Surgeons should not forget to straighten out folds or kinks in the wall of the inserted vessel.

Cost of anastomoses

The cost of the two types of anastomoses was similar, assuming that a single mix of adhesive will provide enough Tisseel for two anastomoses, and assuming that it takes a minimum of two microsutures to complete a standard 1.0 millimetre anastomosis. Compared to the total cost of managing a microvascular patient[334], however, the anastomotic savings would not be important.

Biological aspects

The anastomotic healing process

In Chapter 2 previous descriptions of the healing process of microvascular anastomoses were discussed. In general, investigators have agreed about the main events that take place, although some of the studies varied a little in detail. Differences in experimental design and, in particular, timing of the post-mortem studies, accounts for some of the variations. Other differences are accounted for by the use of different anastomotic techniques, alternate nomenclature, and varying methods for studying tissue specimens.

In this experiment, the healing process of both end-to-end and end-in-end repairs had many features in common and, in

essence, this did not differ greatly from previous descriptions. It probably reflects the normal mechanism of anastomotic healing, which, in summary, consists of:

Immediately after anastomosis there are few biological changes. Technical features relating to the kind of anastomosis used are clearly visible. At this stage, the lumen contains only a small amount of mural thrombus formation at the anastomotic site and at the site of needle punctures.

Within hours, thrombus formation increases in the region of the repair and there is endothelial loss, particularly at the microvascular clamp sites.

By the end of the first week, an inflammatory reaction is present. Early in this process polymorphonuclear leucocytes predominate, but, after about two weeks, these decrease in number, and chronic inflammatory cells became more common. Intramural thrombus progressively declines during the first week, although evidence of re-endothelialisation is not seen till the second or third weeks. During the first week, marked intimal hyperplasia develops at the anastomotic site, the internal elastic lamina fragments near the anastomosis, and areas of hyaline degeneration (with thinning of the vessel wall) occur in the media and inner adventitia in areas strangulated by sutures. At the same time, on the outside of the vessel, there is a fibroblastic reaction which probably represents organisation of haematoma.

By three to four weeks, a smooth layer of endothelial cells covers the lumenal surface, although the intimal hyperplasia remains. At this time, the process of fibrosis and scar formation is well established, although the inflammatory response may not have completely subsided.

In my experiment, differences between end-to-end and end-in-end repairs appeared only to reflect the type of approximation or

method of fixation used to approximate the cut vessel ends. This finding was in agreement with the findings of previous investigators[18,310,412], who believed that the basic healing process remained unaltered regardless of the type of anastomosis used. Wieslander thought that end-in-end and end-to-end anastomoses did differ in their platelet accumulation, organisation, and re-endothelialisation[406,407]. After reviewing his papers, I do not entirely agree with his interpretation, and I feel that his histological sections indicate that there was not a great difference in the two techniques.

Reaction to sutures

The placement of sutures affected the vessel walls in a number of ways. Needle passage caused endothelial damage with resulting mural thrombus formation. Suture material stimulated a foreign body giant cell reaction during the healing phase. Suture tension produced corrugation and bunching of the vessel wall. Suture tension also led to strangulation of tissue. In the sutured anastomoses, the amount of hyaline degeneration observed varied, depending on the location of the section, and was related to the proximity of sutures. Sections near or under sutures were severely affected whilst sections between sutures demonstrated an almost normal histological appearance.

Reaction to adhesive

In the adhesive anastomoses there were no endothelial needle perforations, no foreign body reactions, and there was no evidence of tissue strangulation. There was, however, a response to the adhesive, and the normal appearance of the healing process looked a little different because of the end-in-end configuration of the vascular approximation. The fibrinogen adhesive was clearly identifiable on the histological sections. By the twenty-first day it had all disappeared. During the process of resorption, the adhesive

was surrounded by an intense inflammatory response, although this was neither as great nor as destructive as the one described around cyanoacrylic adhesives. The reason for this inflammatory reaction is not well understood. The fibrin network produced by the adhesive provides a good substrate for the ingrowth of granulation tissue and promotion of wound healing. The normal process of wound healing involves an inflammatory phase and it may be that this is accelerated in the presence of the fibrinogen adhesive. Alternately it has been suggested that the inflammatory response may have been immunological in nature, the fibrinogen adhesive being recognised as a foreign protein. This seems unlikely in view of the type of inflammatory cells observed.

Reaction to overlapping vessel walls

In their long term study of microvascular sleeve anastomoses, Lauritzen et al[206] were concerned that stripping adventitia from the vessels, particularly the inner vessel, might damage the vasa vasorum and interfere with nourishment of the vessel wall. They expected to find focal medial sclerotic lesions as a result of vessel wall ischaemia, but were surprised that the overlapping vessel wall segments were quite well preserved at six months. The same finding was noted in this experiment. Presumably nutrition is obtained either from vasa vasorum that remain intact, or by diffusion from circulating blood, or from rapid revascularisation of the vessel wall.

Aneurysm formation

Aneurysm formation was not a problem with either the sutured or adhesive anastomoses. In 1979, Maxwell et al[247] reviewed the subject of aneurysms after microvascular anastomoses because they had observed a high incidence of aneurysm formation in the rat femoral artery model. They found that the original hypothesis, that interrupted suture technique produced leaks at

the interspace between sutures and subsequent false aneurysm formation, was wrong. Instead they found that aneurysms were true aneurysms, and seemed to be related to mechanical trauma of the vessel walls. The absence of aneurysm formation in this study may reflect either that rabbits were used instead of rats, or that, with the passage of time, microsurgical technique has improved to the extent that vessels are, nowadays, being subjected to less trauma during preparation and suturing[150] of anastomoses.

Mural thrombus

Changes at the cut edge of the inner vessel in the end-in-end anastomoses were examined carefully, because, at this location, the full thickness of the vessel wall was directly exposed to circulating blood. It might be expected that this would stimulate the extrinsic pathway of the blood clotting mechanism (Figure 1), and result in rapid and extensive thrombosis. Sully et al[364] were particularly concerned about this and, although they described their mural thrombi as being non-occlusive, they noted a greater quantity of mural thrombus in their end-in-end anastomoses, and wondered if this could have accounted for poorer patency rates.

In my study, mural thrombus was seen at the inner margin of the end-in-end anastomoses, but this did not result in propagation of thrombus, and did not produce vascular occlusion. The amount of mural thrombus did not appear to differ between the two types of anastomosis compared. The same observation was made by Lauritzen and Hansson[207].

Re-endothelialisation

It is not known how the vascular endothelium regenerates. There are several theories about how it happens:

1. Endothelial cells may regenerate from adjacent endothelium[95,207].

2. Penetrating cells from the media may differentiate into new endothelial cells[384].
3. Multipotential cells from the blood stream may settle and differentiate into endothelial cells[114].

The investigations carried out in this experiment were not aimed at detecting the origin of the new endothelial cells and did not really throw any new light on this aspect.

Previous investigators have described the timing of endothelial regeneration (see page 64). Their results have indicated that re-endothelialisation may take from one to four weeks. The time variation probably represents interpretation of experimental results (observations being made at different times, depending on experimental design) rather than true variation in re-endothelialisation. Nightingale et al[268] made observations at frequent time intervals, and their description indicates that re-endothelialisation is virtually complete at two weeks, but it takes nearly four weeks for correct cellular axial alignment to be achieved. The observations made in my study concur with Nightingale's findings.

Re-endothelialisation of end-in-end anastomoses seemed to be slightly slower than end-to-end anastomoses. It is possible that this might represent observer variation, but it might also indicate that it takes longer to re-endothelialise a wider gap. Previous investigators have demonstrated that it takes longer to re-endothelialise sutures than the anastomotic margin in end-to-end anastomoses.

Cellular hyperplasia

Cellular hyperplasia was seen in both end-to-end and end-in-end anastomoses, although it was greater in the end-in-end anastomoses, where there was a larger gap to be filled at the inner margin of the sleeve. The 'flow' of cells appeared to smooth the

inner lining of the vessel in a way that would again promote laminar flow. Chen et al[64] speculated that the intimal hyperplasia might be related to changes in the dynamics of the bloodstream, and Meyermann et al[253] also suggested that 'the whirling blood flow' of end-to-side anastomoses produced more intimal thickening.

The nature and origin of these cells was not elucidated in this study. Theoretically, because changes at the anastomotic site were part of a normal healing process, it would not be surprising if these cells were myofibroblasts. The hyperplastic cells were located between the intima and the internal elastic lamina. Khodadad[184] called the hyperplasia 'intimal' because it was located lumenal to the internal elastic lamina. Baxter et al[26], seeing it in the same location, called it 'subintimal' because they theorised that the hyperplasia was the result of smooth muscle cells migrating from the media. They considered the hyperplasia to be a normal healing response and stated that the media was the most important mural component in the repair of small vessels. Chen et al[64] did not agree with Baxter et al. Noting that muscle cells of the media disappeared in the region of the anastomosis, they felt that the hyperplastic cells originated from elsewhere, and suggested that they might represent fibroblasts differentiating into smooth muscle cells as a result of injury. Lidman and Daniel[215] called the proliferating cells 'myointimal cells' and described them as a 'multipotential mesenchymal repair cell with capability to form collagen, elastic fibres and also smooth muscle filament, all depending on the demand of the situation'. This suggests that they thought the cells were myofibroblasts.

The best way to determine if the cellular hyperplasia was made up of myofibroblasts would be to use transmission electron microscopy. The literature contains very little about this method for studying microvascular anastomoses.

Blair et al, in their histological paper[34], briefly mentioned some transmission electron microscopic observations. They do not

say that they saw myofibroblasts, but do say that 'smooth muscle cells were in a state of high intracellular activity in Zone 2b', which is where the maximal amount of intimal hyperplasia is located.

Meyermann et al[254] described two ultrastructural changes in the intima after microsurgical operations:

1. Widening of the subendothelial space.
2. An increase of specific organelles (tubular bodies, which are thought to be related to the blood coagulation system).

Unfortunately, Meyermann's study[254] concentrated on changes in the endothelium, and does not mention whether or not myofibroblasts were seen in the hyperplastic region.

Ts'ao[384], in his study of damaged abdominal aortae, suggested that the endothelium is replaced by 'myointimal cells', which migrate from the media to the intima. It is not clear whether these cells were true myofibroblasts, but from his description it looked as if they might have been.

Lauritzen and Hansson[207] made transmission electron microscopic observations of the area between the two layers of sleeve anastomoses. At one week they saw macrophages, cell debris, and invading cells 'resembling reactive fibroblasts and smooth muscle cells'.

Taking all this information together, it seems likely that the hyperplastic cells are myofibroblasts, a conclusion that would fit in with current theories of wound healing.

CONCLUSIONS

The experimental study has demonstrated that the fibrinogen adhesive, Tisseel, can be used to make a successful end-in-end

microvascular anastomosis. Anastomotic patency rates were comparable to conventional sutured anastomoses, but had advantages in that they could be completed more easily and in less time. The disadvantage of the adhesive technique is that it is less versatile, but it does provide an additional to the anastomotic armamentarium that may be useful in a number of clinical situations (see page 187).

The histology and SEM appearances of the two types of anastomoses have demonstrated the normal process of anastomotic healing, and differentiated factors that are part of the normal process, from factors that are related to the type of anastomosis or method of fixation.

CHAPTER 6

The Role of Adhesives and the Future of Microvascular Surgery

INTRODUCTION

The experiment described in Chapter 5 demonstrated that a series of end-to-end microvascular anastomoses, carried out experimentally in the rabbit femoral artery, will produce patency rates and a healing process comparable to conventional suture anastomoses.

Chapter 6 begins by examining the advantages and disadvantages of adhesive microvascular anastomotic technique in order to place its possible clinical application into perspective. Next, there is a discussion of some other uses that fibrinogen adhesive may have in Microvascular Surgery. The chapter concludes with a look at some possible future applications of Microvascular Surgery.

ADHESIVE MICROVASCULAR ANASTOMOSES

The preceding chapters have reviewed the development and contemporary role of microvascular surgery and hence the indications for microvascular anastomoses. A large variety of techniques for making microvascular anastomoses have been described. Some of these are commonly used, whilst others are little more than research curiosities.

Adhesive microvascular anastomoses fall into two general categories: those made with synthetic adhesives and those made with biological adhesives.

The synthetic adhesives have some advantages in that they do not involve the use of human blood products and they do not need to be mixed at the time of operation. Their main technical disadvantage is that they are highly inflammatory and cause damage to adjacent soft tissues. As a result anastomotic patency is unpredictable, at best and, in general, does not compare favourably

to the 'gold standard' sutured anastomosis. This disadvantage, together with the possibility that they may induce soft tissue sarcomas, has precluded them from clinical use.

A biological fibrinogen adhesive is not locally detrimental to adjacent tissue and has a number of advantages. In addition to functioning as an adhesive for the small blood vessels being anastomosed, it offers other qualities that may be beneficial to the operative site. It may reduce haemorrhage, and it may promote healing. Another advantage is that it may be a useful adjunct during other stages of the operation. For instance it may be used for the repair of peripheral nerves, for the application of skin grafts or as an adjunct when packing a cavity with morcellised cancellous bone graft. The main disadvantage of fibrinogen adhesive is the risk of disease transmission, but this appears to be more theoretical than real. Other disadvantages might include the inability to use the described anastomotic technique for end-to-side and size discrepancy anastomoses.

In practical terms, the commercially made fibrinogen adhesive, Tisseel, has been marketed and packaged with a great deal of care, and close attention has been paid to user education. This has resulted in a product that is easy to use and that has minimal waste. Mixing, in theory a complex process, has been made simple, and the Duploject system has made application a very straightforward process.

Suturing is still the most common method for securing a microvascular anastomosis. The technique is versatile and can be used for all types of anastomoses. Suture technique can be used in a variety of different ways. The theoretical disadvantage of suture technique is that inaccurate suturing can damage the intima at the site of needle entry and introduce irregularity to the intra-lumenal surface. In practice, contemporary microvascular surgeons are technically skilled, and modern suture materials are so well made that these problems are not a major issue. Suture technique does,

however, require the use of a high degree skill and intense concentration over a prolonged period of time. This leads to fatigue.

The experiment described in Chapter 5 demonstrated that fibrinogen adhesive anastomoses remain patent and can support cutaneous flaps with results that were comparable to conventional sutured anastomoses. The fibrinogen adhesive anastomoses can be secured more rapidly and are, subjectively, less technically demanding than sutured anastomoses. Fibrinogen adhesive end-in-end anastomoses are not as versatile as sutured end-to-side anastomoses. An end-to-side technique has not been developed, and use of the technique for venous and size discrepancy anastomoses has not yet been investigated. There may be situations when a fibrinogen adhesive anastomosis has advantages over a sutured anastomosis. For instance, fibrinogen adhesive might be very useful for the awkward anastomosis situated in a deep intermuscular cleft where the microvascular clamp cannot be rotated, particularly if blood is pooling in the same location and causing loss of visibility. The fibrinogen adhesive can be used for haemostasis by applying it directly to a raw bleeding surface or to make a temporary dam of fibrin prior to completing the anastomosis. Another use for a fibrinogen adhesive anastomosis might be in the difficult 'vertical' anastomosis sometimes encountered in lower limb reconstructions. It may be easier to complete an adhesive end-in-end anastomosis than a sutured end-to-end anastomosis in this circumstance.

Some surgeons may wish to use the fibrinogen adhesive anastomosis as their standard anastomotic method. In the final analysis, however, it may be that the main advantage of the fibrinogen adhesive anastomosis lies in the subjective finding that it is technically easier to carry out than suturing and demands less surgical expertise. The technique might be particularly useful for difficult replantations. For instance in an amputation at the level of

the distal palm or proximal part of the digits, especially when there are multiple digits involved, a replantation or revascularisation can be expected to take many hours to complete. Fatigue can be minimised by using a team approach, but these operations are frequently carried out in the middle of the night or on weekends, when fewer team members are available. Fatigue may become a serious problem. If fatigue leads to inaccuracy in microsurgical suture technique, anastomoses may be damaged and/or fail. Unsatisfactory anastomoses have to be revised, which prolongs the operation, increases fatigue and ultimately may result a failed reconstructive procedure. In these circumstances the less technically demanding adhesive technique might be particularly useful.

OTHER USES FOR ADHESIVE IN MICROVASCULAR SURGERY

Although experience with the use of fibrinogen adhesive in Microvascular Surgery is still in its infancy, the results of the experiment described in Chapter 5 indicate that adhesive would not enter the vascular lumen even if was applied in quantity directly onto the anastomosis. This suggests that it would be safe to use fibrinogen adhesive in the wound during a microsurgical operation. Thus, in addition to making the microvascular anastomoses, fibrinogen adhesive could be used for a number of other functions during a microvascular operation.

Haemostasis

Persistent bleeding may be encountered during microvascular operations. It can arise from a variety of sources such as dissection through scar tissue, 'freshened' granulation tissue, anastomotic leakage, or anticoagulation, which is sometimes used in difficult

cases. Bleeding can be associated with a number of problems. For instance:

1. A continuous ooze throughout a long operation will result in loss of blood volume. If this can be minimised, there will be maintenance of blood pressure, with better tissue perfusion, and less need for transfusion, which will reduce likelihood of transfusion complications.

2. Bleeding from tissue in the area where a microsurgical anastomosis is being made can be particularly troublesome. It may produce extreme technical difficulties by preoccupying the surgeon, or his assistant, and/or obliterating the field of vision. The result of this situation may be a poor anastomosis and subsequent flow problems.

3. After the anastomoses have been completed and the flap has been inset into the wound, unsatisfactory drainage, associated with persistent bleeding, can result in haematoma formation in the dead space around the anastomoses. This problem can cause vascular compression and an extra-lumenal flow obstruction. In order to salvage the failing flap, the patient must be returned to the operating room, have the haematoma evacuated, haemostasis secured, and frequently the anastomoses revised. Ischaemic reperfusion injury is a serious problem associated with this situation.

Colen and Mathes[76] experimented with the use of microcrystalline collagen for controlling haemorrhage in the region of microvascular anastomoses. They reported that it did not adversely affect patency rates even when applied directly onto the anastomosis. The results of my study suggest that fibrinogen adhesive could be used in the same way for the reduction of haemorrhage in microvascular operations.

Nerve repair

The use of fibrinogen adhesive for making microneural anastomoses was discussed in Chapter 3. Fibrinogen adhesive is commonly used for making microneural anastomoses in some parts of the world. This technique can be used in conjunction with microvascular repair, and its use should be considered in replantations and free tissue transfers using composite tissue transplants (e.g. functional muscle transplants; neurovascular flaps) that include nerve[244].

Bone replacement

The use of fibrinogen adhesive as an adjunct to morcellised cancellous bone grafting was discussed in Chapter 3. This technique might be useful for filling in small bone defects during replantations[244,332].

Pedicle kinking

Kinking of the vascular pedicle can be a troublesome problem, especially if the length of the vessels is not perfect. This problem can be minimised by removing retractors and carrying out a 'dress rehearsal' positioning the flap and pedicle prior to completing the microsurgical anastomoses. Despite this precaution, however, vessel length can still be inaccurate and kinking can result from vascular redundancy. It is important to place the anastomoses and vessels in a gentle curve and to locate them away from sharp edges, which may arise in the vicinity of cut fascia or metallic fixation devices. Cardiovascular surgeons have used fibrinogen adhesive to fix the position of coronary arteries to prevent kinking[316]. This application has not yet been reported in the microsurgical literature, but there is no reason why this technique could not be used in Microvascular Surgery. It may be a better solution than the use of a fixating microsuture, which itself may lead to kinking.

Dead space

As mentioned above, there is always a certain amount of dead space under a flap, and this is usually located around the vascular pedicle. It is important to thoroughly drain the wound deep to the flap so that pools of blood cannot form haematomas that may lead to pedicle compression. This is particularly important in the anticoagulated patient. Drainage is the best solution to this potential problem, but filling the dead space by 'gluing down' the flap with fibrinogen adhesive would also be a useful preventative measure.

Skin grafts

The use of fibrinogen adhesive as an adjunct to split thickness skin grafting was discussed in Chapter 3. This technique has been used partly because fibrinogen adhesive is believed to promote wound healing. Rather than using cutaneous or myocutaneous free flaps for defects in the lower leg, there is now a tendency to use free muscle transfers covered with split thickness skin grafts. The use of fibrinogen adhesive to attach the skin grafts would save time at the end of the operation.

FUTURE DEVELOPMENTS IN MICROVASCULAR SURGERY

Future developments in Microvascular Surgery cannot be separated from a discussion of microvascular anastomoses because the two are mutually inseparable.

It is likely that microvascular technique will become used more frequently as free tissue transplantation increases in scope and sophistication. Surgeons will continue to strive to find faster, simpler and better ways to anastomose small blood vessels in order to facilitate the ease with which operations can be carried out. It is likely that investigators will continue to examine many different

techniques, and it is probable that adhesive microvascular anastomoses will find a niche in the overall armamentarium of techniques available for the microsurgical repair of small blood vessels.

In 1965, Fisher[104] first reported on the use of microvascular surgical techniques in experimental organ transplantation. Since that time, the possibilities for both experimental and clinical tissue transplantation have increased immensely, largely because of major advances in the field of immunology.

The development of cyclosporin A has been associated with increasing success in the transplantation of vital organs such as kidney, liver, bone marrow and heart. It has even facilitated transplantation of the lung, which is highly antigenic. The improved results and relative safety of this drug have encouraged reconstructive microvascular surgeons to look further into the possibility of allograft transplantation of non life-maintaining tissues.

The possibility of using allograft transplantation procedures for reconstructive hand surgery is particularly attractive. Despite the spectacular success of replantation, there are still many patients whose injuries are so devastating that their final outcome is less than satisfactory. The ultimate dream would be to have a tissue bank containing numerous tissues, varying in size, shape and cosmetic appearance, which could be used for all kinds of free tissue transfer operations.

There are many differences between the vital parenchymal tissues, whose transplantation has now become common, and the type of tissues used for extremity reconstruction. At the present time there appear to be two major differences, and these have been the focus of academic discussion and laboratory research:

1. The variety of different tissues in the limbs and the considerable variation in their degree of antigenicity (unfortunately, skin presents the immune system with a particularly great challenge).
2. The absolute need for re-innervation that is required if satisfactory functional results are to be obtained in the extremities (it seems that visceral transplants can function adequately without direct neural regulation).

There are a number of reports describing experimental limb transplantation[91].

Some whole limb transplants have been carried out using methods that would not be possible in humans. Lapchinsky[250] used a technique involving an exchange transfusion from donor to recipient dogs, several months prior to transplantation, to achieve immunological tolerance. The method of parabiosis, by the surgical union of two very young individuals, was examined by Schwind[333]. Poole et al[308] were able to induce tolerance, prior to transplantation in adults, by first carrying out an antiserum-enhanced renal allograft transplant.

Other investigators have examined the effect of different drug induced immunosuppression regimens. In the days prior to the use of cyclosporin A results were far from ideal. In a canine experiment[122] the use of 6-mercaptopurine and azothioprine were shown to slightly prolong graft survival although they did not prevent rejection. Results were not very different in a similar experiment carried out by Doi[96] using rat limb transplantations. In both these studies, side effects of the drug regimen proved to be fatal on numerous occasions. One study[200] did obtain prolonged survival of canine limb transplantations, but this was only achieved with the use of a very potent combination of multiple

immunosuppressive therapies, and severe side effects were encountered.

In the 1980s experiments with limb transplantation have used cyclosporin A for immunosuppression. A number of publications concerning adult rat limb transplantation[31,109,154] have demonstrated that cyclosporin A can dramatically improve transplant survival. In many of the experimental animals there was no histological evidence of rejection, a finding that differed markedly from the histological observations made in the non immunosuppressed controls. Kim et al[186] compared adult rat hind limb allograft transplantations with a replantation control. Their results indicated that transplantations in rats treated with cyclosporin A behaved similarly to replantations.

Following these reports, demonstrating the striking improvement in the survival of composite tissue transplantation when cyclosporin A was used for immunosuppression, investigators have gone on to examine the results of similar experiments in primate species. Particular emphasis has been placed on the return of nerve function, partly because this is so important if satisfactory limb function is to be achieved and partly to ensure that cyclosporin A does not in some way (its mode of action is not yet clearly defined) inhibit functional re-innervation.

In 1986, Daniel et al[91] described an experiment in which four hand transplantations and seven neurovascular free flap transplantations were carried out in baboons immunosuppressed with a combination of cyclosporin A and methylprednisolone. Stark et al[359,360] have subsequently reported the results of similar experiments in which baboon hand transplantations were carried out using cyclosporin A and prednisolone. The results of both groups of investigators clearly indicated that limb transplantation can be achieved in primates, and that recovery of motor and sensory function will occur in hand transplantation in a manner that is comparable to replantation.

The reason that limb allografts are still not carried out clinically is that, even with cyclosporin A, the toxicity of the required immunosuppression is too high for elective reconstructive surgery. Cyclosporin A is nephrotoxic at high levels and hepatotoxic when combined with other immunosuppressants. In the primate experiments[91,359,360] it was necessary to obtain high serum levels of cyclosporin A to ensure survival of the limb allografts. The dominant target of rejection appeared to be the skin, whilst the skeleton and other soft tissues were better tolerated. It has been suggested that hands could be transplanted denuded of skin and then covered with recipient skin grafts. The best solution, however, will be the development of more selective, more effective and less toxic immunosuppressive drugs.

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APPENDIX

APPENDIX. Papers written and directly related to this thesis.

Papers published:

1. Bowen CVA. Reconstructive microsurgery: Historical background, application and techniques for small blood vessel repair. Transplantation Implantation Today 5:37-43, 1988.

Papers submitted for publication:

1. Bowen CVA, Leach DH, Crosby NL and Reynolds R. Microvascular anastomoses. A comparative study of fibrinogen adhesive and interrupted suture techniques. (Plast Reconstr Surg).

2. Bowen CVA, Leach DH and Crosby NL. A comparison of the healing process in two techniques of microvascular anastomosis. (J Reconstr Micro).

Papers commissioned for publication:

1. Bowen CVA. History of microvascular anastomotic technique. I. A review of suture techniques. (Microsurgery).

2. Bowen CVA. History of microvascular anastomotic technique. II. A review of non-suture techniques. (Microsurgery).